THE DISSERTATION ON

"BACTERIAL, FUNGAL AND PARASITIC INFECTIONS IN PATIENTS ON HEMODIALYSIS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN A TERTIARY CARE HOSPITAL"

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CERTIFICATE

This is to certify that the dissertation entitled **"BACTERIAL, FUNGAL AND PARASITIC INFECTIONS IN PATIENTS ON HEMODIALYSIS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN A TERTIARY CARE HOSPITAL"** is the bonafide work done by **Dr.R.SYNTHIA SELVAKUMARI,** during her M.D. Degree Branch – IV (Microbiology) is a bonafide research work carried out by her under the direct supervision & guidance of

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DECLARATION

I, **Dr.R.SYNTHIA SELVAKUMARI**, declare that, I carried out this work on **"BACTERIAL, FUNGAL AND PARASITIC INFECTIONS IN PATIENTS ON HEMODIALYSIS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN A TERTIARY CARE HOSPITAL"** is submitted by me for the degree of M.D, the record work carried out by me during the period of September 2011 to October 2012 under the guidance of **Dr.G.JAYALAKSHMI, MD., DTCD.,** Director and Professor of Microbiology at the Institute of Microbiology, Madras Medical College, Chennai. I also declare that this bonafide work or a part of this work was not submitted by me or any other for any award, degree or diploma to any other University, Board, either in India or abroad.

This is submitted to the Tamilnadu Dr.M.G.R.Medical University, Chennai in partial fulfillment of the rules and regulations for the M.D Degree examination in Microbiology.

Dr. R. SYNTHIA SELVAKUMARI

Place : Chennai
Date :

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INTRODUCTION

Hemodialysis improves the quality of life in patients of end stage renal diseases. Hemodialysis(HD) is a type of machine with an artificial filter which removes wastes from the blood stream and regulates the body fluid mechanism and chemical balances. Vascular access acts as bridge between the patient and machine circuit hence it is called the Achillis heal of Hemodialysis[1,2].

In modern medicine, the use of temporary intravascular catheters for vascular access and haemodynamic monitoring has become a central part in patients undergoing haemodialysis. Central venous catheters (CVC) have significant benefits in many clinical situations but the major consequence of t CVC is colonization of the catheter by either bacteria or fungi, which can lead to catheter related infection (CRI) and serious catheter related blood stream

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In modern medicine, the use of temporary intravascular catheters for vascular access and hemodynamic monitoring has become a central part in patients undergoing hemodialysis. Central venous catheters (CVC) have significant benefits in many clinical situations but the major consequence of CVC is colonization of the catheter by either bacteria or fungi, which can lead to catheter related infection (CRI) and serious catheter related blood stream infection (CRBSI) which remain a major cause of nosocomial infections leading to significant patient morbidity, mortality and hospital costs[1,2,3,4] The frequency of hemodialysis is a risk factor for infection.

Patients with renal impairment are at high risk of developing infection due to high uremic state, low immunity, severe clinical deterioration and need vascular access for Renal Replacement Therapy (RRT).[5] The risk of developing bacteremia varies with the site of CVC insertion, type of device and duration of CVC in situ.[6]

CRBSI have increased in incidence during the past few decades. More than three fourth of the nosocomial bacteremias occurring in case clusters are

primary bacteremias and the case fatality rate of CRBSI has been estimated about 10-20% and more than 90% of CRBSI are associated with CVC[7,8].

An estimated 248000 Blood Stream Infection (BSI) occur in United State Hospital each year[9]. More than 25000 patient develop device related bacteremias in US each year[7].

The United States Centers for Disease Control (CDC) National nosocomial infection survey indicates that 82% of all episodes of nosocomial bacteremias occurring the survey hospitals and presence of CVC is the single biggest risk factor for BSI among HD patients[1,10].

In early 1970's HD was used all most exclusively for Acute Renal Failure(ARF). The vascular access devices particularly Internal Jugular Vein (IJV), femoral vein and subclavian vein are major source of infection, causing local and systemic infection like local cellulitis, abscess formation, septic thrombophlebitis, endocarditis and septicemia in the HD patients[11,12]

HD patients are at a higher risk of developing fungal infections by pathogenic and opportunistic fungi due to the defective immune system[13]. In an immunosuppressed individual parasitic infection can cause profuse diarrhea, anorexia, malabsorption syndrome and weight loss. Accumulation of non excreted metabolites by kidney and chronic renal insufficiency leads to uremia and induces a state of immunosuppression that results in higher frequency of aquring bactieral, fungal and parasitic infection through CVC, contact with nursing staff, equipment, material on surface and from hands[14].

CDC's health care infection control practices advisory committee CDC/HIPAC guidelines for prevention of IVC related infection 2011 gives the guidance for prevention of Central Line Associated Blood Stream Infection (CLABSI)[15].

All these infections are reversible and in view of the significant morbidity and mortality among patients with CVC, early diagnosis, management and prevention of such infection is mandatory.

This study was undertaken to determine the incidence of bacterial, fungal and parasitic infection in patient on hamodialysis with Central Venous Catheter, to analyze the associated risk factors and to perform antibacterial and antifungal susceptibility pattern. This study was done in the Institute of Microbiology in association with Department of Nephrology, Madras Medical College, Rajiv Gandhi Government General Hospital, Chennai.

REVIEW OF LITERATURE

HISTORY OF DIALYSIS:

1913 The term artificial kidney was coined by ABEL, ROWNTUE, TURNER at JOHNS HOPKINS in BALTIMORE. They devised a kidney using celloidin tubing for membranes and used crushed leach heads (Hirudin) for anticoagulation. The kidney worked inefficiently and the experimental animals died of hypersensitivity to hirudin.

1923 N1CHIOLES in Germany used peritoneal membrane from an ox to make artificial kidney.

1943 WILLEM KOLFF designed the rotating drum artificial kidney. It was used successfully in Holland, to treat patients with kidney failure. KOLFF published his design in 1946

1947 KOLFF compared the effectiveness of peritoneal and intestinal lavage to hemodialysis and found hemodialysis superior for urea removal.

1947 VONGARRETTS in COPENHAGEN made a hand wound coil kidney and used it successfully in humans.

1953 TESCHAN and colleagues used artificial kidneys to treat battle injuries with acute renal failure in Korean hospitals. The survival of patients with battle injuries and acute renal failure was improved.

1956 KOLFF developed a disposable coil dialyzer.

1957 KIIL developed the flat plate paralleled flow dialyzer.

1959 SCRIBNER started the first two patients on chronic dialysis using the KIIL dialyzer.

1964 Home dialysis was started by CURTIS and SCRIBNER .

1965 The subcutaneous arterioverous fistula was developed by BRESCIA and CIMINO.

1969 First used subclavian catheters by ERBENEL

1974 Large surface area dialyzers became available that allowed a decrease in dialysis time.

1979 ULDALL introduced Modified single needle dialysis cannula.

1980 Automated peritoneal dialysis and Continuous ambulatory peritoneal dialysis widely accepted for chronic maintenance dialysis.

1983 GRABE used inner surface of the cannula for semiquantitative roll plate culture of steel stiletto inserted in the lumen and drawn back and forth.

1984 BOZETTI was used nonquantitative culture of catheter blood.

1985 COOPER performed Direct Grams staining of the catheter segment

1990 CERCENADO used semiquantitative catheter tip for colony count

1992 CAPDEVILA introduced paired and unpaired quantitative blood culture methods. READ *et al* and GUTIERREZ *et al* used quantitative culture of catheter segment

1993 KHARDORI introduced culture of catheter segment embedded into agar.

1993 RUSHFORTH used catheter blood for Acridine Orange staining of monolayered leucocytes from pellet examining with UV microscope

2001 Infectious disease society of America (IDSA) published a Clinical practice Guidelines on management of Intravascular catheter related infection.

2008 Europian Renal Best Practice (ERBP) gives Guidelines for the exclusive management of hemodialysis.

2009 Infectious Diseases Socity of America (IDSA) published an update of Clinical Practice Guidelines for the Diagnosis and Management of Intravascular catheter related infection.

2011 CDC's Healthcare Infection Control Practices Advisory Committee (CDC/HIPAC) released Guidelines for the prevention of Intravascular Catheter related Infection

Acute hemodialysis access is through Internal Jugular, Subclavian and Femoral vein catheterization, using double lumen catheters.

End stage renal disease is a complete failure of ultra filtering capacity of kidneys which is a complication of many diseases as a result of the accumulation of metabolic byproducts, loss of controlling functions of kidneys on electrolytes, disturbance of several of the endocrine functions of kidney and ultimately leading to death[16]. The definite solution of the end stage renal disease is transplantation but not all the patients are suitable for transplant. Therefore the alternative is dialysis procedure which is based on circulating blood on one side of the semi permeable membrane against dialysis solutions on the other. Thereby they constantly remove the accumulated metabolic degradation products from the blood stream[17].

INCIDENCE OF HEMODIALYSIS ASSOCIATED INFECTION

In practice, one of the most frequent and serious complications of using CVC devices has been the development of infections. 400,000 episodes of vascular catheter related blood stream infections occurs in USA every year[16]. Infectious complications associated with the use of these devices range from 2.7% to 21%. More than 3/4 of the nosocomial bacteremia occurring in case clusters are primary bacteremia and more than 75% of these are device related[7]. CRBSI is reported to be 2.5 – 5.5 cases per patient year. Canadian Nosocomial Infection Surveillance Program found that the risk of bacteremia is higher in cuffed or uncuffed CVC than Arterio Venous Fistula(AVF).[6,23] Enhanced susceptibility secondary to the alterations in the immune response induced by uremic state play a major role in determining the type, incidence and outcome of infectious complications[18]

Thoburu *et al*, and Ratnaja *et al* noted that septic thrombophlebitis in patients with central venous catheters may account for 20 % of hospital acquired septicemias.[6,19,20] Bacteremia during hemodialysis has been reported with an incidence of 0.15 episode per patients dialysis year[21,22].The mean incidence of CRBSI for 'temporary –untunnelled' catheters was reported as 5.0 episodes/1000 catheter days and 3.5/1000 catheter days for permenant – tunneled cuffed catheters[23]. CRBSI has been reported about 15% in Mumbai , India.[2]

HEMODIALYSIS MODALITIES:[24,25]

a. HEMODIALYSIS:

1. Conventional
2. Sequential Ultrafiltration / Clearance
3. Ultrafiltration - Central Venovenous, Central Arteriovenous

b. HEMOFILTRATION:

1. Slow continuous ultrafiltration
2. Continuous Arteriovenous Hemofiltration
3. Continuous Arterio venous Hemodialysis
4. Continuous Arteriovenous Hemodiafitration
5. Continuous Venovenous Hemofiltration
6. Continuous Venovenous Hemodialysis
7. Continuous Venovenous Hemodiafiltration

INDICATIONS OF HEMODIALYSIS:[24,25,26]

A. ACUTE DIALYSIS:

1. Uremic Syndrome
2. Hyperkalemia
3. Acidosis
4. Fluid Overload
5. Acute Renal Failure
6. Organo Phosphorus Poisoning
7. Acute Pulmonary embolism
8. Cerebro Vascular Accident
9. Congestive cardiac failure.

B. CHRONIC DIALYSIS:

Chronic Renal Failure

End stage Renal disease

C. OTHER INDICATIONS:

1. Cirrhosis of Liver
2. Guillian Barre Syndrome
3. Hypokalemic Periodic Paralysis
4. Chronic Corpulmonale
5. Pericardial effusion
6. Myxoedoma coma
7. Status Epilepticus
8. Acute Myocardial infarction
9. Cardiogenic Shock

10. Drug Interactions
11. Hypercalcemia
12. Hypothermia
13. Hyperuricemia
14. Metabolic Alkalosis

ACCESS FOR HEMODIALYSIS: [27]

Site from where the blood was taken out from the body and then returned to the blood stream is called Access. The various access modalities are Fistula, Graft and Catheter.

Central venous catheter: Short term if the catheter was in place for less than 7days, Long term if it was more than 7 days

1. PERCUTANEOUS VENOUS CATHETERS:

a. Femoral Vein

b. Subclavian Vein

c. Internal Jugular Vein

2. EXTERNAL ARTERIO VENOUS SHUNT:

Radial Artery-Cephalic Vein

3. ARTERIOVENOUS FISTULA:

A. ENDOGENOUS:

Radial Artery-Cephalic Vein

Ulnar Artery-Brachiocephalic Vein

Brachial Artery- Brachiocephalic Vein

Femoral Artery-Saphenous vein

B. PROSTHETIC:

Dacron

Polyurethane

Polyethylene

Polytetrafluroethylene

TYPES OF VASCULAR ACCESS

Acute	Chronic
External Arteriovenous Fistula	Native Arteriovenous Fistula
Double Lumen Catheter	Synthetic Arteriovenous Fistula

COMPLICATION OF HEMODIALYSIS: [6,25]

Acute	Chronic
Pyrogenic Reaction	Inadequate Clearance
Hypotension	Beta 2 Microglobulin Amyloidosis
Anaphylaxis	Aluminium Intoxication
Arythmias	Accelerated Atherosclerosis
Bleeding	Hypertrophic Cardiopathy
Hypoxemia	Infective endocarditis
Cramps	Septic Arthritis
Hemolysis	Septic Embolism
Dialysis Disequilibrium Syndrome	Osteomyelitis

The development of infections in hemodialysis with the devices depend on the organism and dose, structure of the device material, fibrin, fibronectin and platelet deposits on the device and route of infection. 73% of bacteremia

are due to access site infection.[28] The surface of all devices contains microscopic irregularities which may trap fibrin, platelets and bacteria.

Central Venous Catheter Related Infections are considered under two headings: They are

LOCAL CATHETER INFECTIONS:

a. EXIT OR INSERTION SITE INFECTION:[29] It is considered major portal of entry of microbial access leading to catheter related sepsis. Micro organisms grow proximally along the outside of the catheter, enter circulation via intravascular portion of catheter and cause sepsis. Presence of edema, erythema, thrombophlebitis or exudate formation around insertion site are the signs of infection.

b. TUNNEL INFECTION: Spreading cellulitis around subcutaneous tract of long term tunelled catheter is termed as tunnel infection[7]

c. CATHETER COLONIZATION: In the absence of clinical signs of infection at the exit site, < 15 CFU on quantitative culture or < 15 CFU on semi quantitative culture of catheter segment is termed as catheter colonization.

SYSTEMIC CATHETER RELATED SEPTICEMIA:

It may be of two types,

a. PROBABLE CATHETER RELATED SEPSIS OR PRIMARY SEPTICEMIA: Blood Stream infection caused by organisms from the skin of patients

with vascular catheters who have manifestation of sepsis and no apparent source of septicemia except the catheter.[29,30]

b. DEFINITE CATHETER RELATED SEPTICEMIA: First degree septicemia for which there is clinical or positive quantitative culture implicating catheter as the source of sepsis.

Criteria for systemic catheter related septicemia

1. Pus at the insertion site: Isolation of the same organism from pus and blood stream.

2. Clinical sepsis which is refractory to antibiotics but which resolves after removal of catheter.

3. Positive quantitative catheter culture with the isolation of same organism from the catheter and blood stream.

Differential quantitative blood culture > 10 fold increase in colony count of organisms isolated from the blood drawn through central catheters verses that simultaneously drawn from the peripheral venous blood [29]

EPIDEMIOLOGY

RISK FACTORS FOR INTRAVASCULAR CATHETER INFECTIONS:

I. HOST RELATED FACTORS:[6,7,26,]

1. Elderly Patients
2. Anaemia
3. Hypoprotenemia

4. Diabetes Mellitus

5. Recent hospitalization

6. Recent surgery

7. History of previous dialysis

8. Peripheral atherosclerosis

9. Personal hygeine

10. Nasal carriage of Staphylococcus aureus

11. Alteration on cutaneous flora

12. Granulocytopenia

13. Immunosuppresive therapy

14. Loss of skin integrity eg: burns, psoriasis
15. Severity of the underlying illness
16. Presence of distant infection
17. High cumulative dose of intravenous iron.
18. Array of diagnostic and theraputic modalities.

II. ENVIRONMENTAL RISK FACTORS:

1. Type of catheter- plastic , steel
2. Location of catheter- central, peripheral, femoral subclavian
3. Duration of placement of catheter
4. Type of procedure - emergency, elective

5. Skill of the venipuncturist
6. Contaminated skin antiseptics
7. Moisture around the dressing
8. Tight or occlusive dressing
9. Improper aseptic techniques
10. Occurrence of clot at the tip of fibrin sleeve, insertion length.
11. Maintenance of hygiene by the Health care provider - hand washing

III. ADDITIONAL RISK FACTORS:

1. Catheter composition /construction / flexibility / stiffness
2. Microbial adherence properties - Hematogenous seeding of catheter
3. Biofilm formation

ETIOLOGICAL AGENTS

SPECTRUM OF CATHETER RELATED BLOOD STREAM INFECTION [7,12,14,31,35-43,46]

BACTERIAL

Staphylococcus aureus	-	Acinetobacter species
Staphylococcus epidermidis	-	Enterobacter species
Corynebacterium jeikeium	-	Klebsiella species
Bacillus species	-	Citrobacter species
Pseudomonas aeruginosa	-	Micrococcus species
Stenotrophomonas maltophilia	-	Enterococcus Species
Aeromonas species	-	Escherichia coli

Serratia marcescens - Polymicrobial

ATYPICAL MYCOBACTERIA:

Mycobacterium fortuitum

Mycobacteriurn chelonae

FUNGAL:

Candida albicans

Candida parapsilosis

Candida tropicalis

Malassezia furfur

Rhodotorula species

Fusarium species

Trichosporon species

PARASITIC:

Blastocystis hominis

Cryptosporidium parvum

Isospora belli

Endolimax nana

Entamoeba coli

Entamoeba hartmanni

Dientamoeba fragilis

ROUTES OF TRANSMISSION[31]:

1. Intraluminal spread-contaminated entry port, contaminated fluid

2. Extraluminal spread-hospital staff or patients micro flora, improper aseptic technique

3. Hematogenous spread
4. Other routes - furniture, bed clothes, intensive care unit, contaminated medical device, antibiotic resistance, suppurative thrombophlebitis

PATHOGENESIS

The balance of forces between two competing life forms, the human host and the infecting microbe is popularly known as 'EQUATION OF INFECTION'[7] Indwelling devices heavily bias the infection equation in favour of the parasite and enable non pathogenic bacteria to opportunistically infect foreign body. They cause chronic inflammation which cripples the ability of host to opsonize and phagocytose bacteria. [7,32].

There are three interactions in the pathogenesis of intravascular infections such as:

a. Effect that the host has upon the medical device

b. Effect that the medical device has upon the pathogen

c. Effect that the pathogen has on the medical device[7] The striking feature of indwelling medical device is the diversity in function, design, construction and the location of these appliances.

INTERACTION BETWEEN DEVICE AND HOST:[7]

The magnitude of tissue response is influenced by the chemical composition, shape and mechanical stability of the implanted object e.g. very few plastics are chemically inert. The warm, wet and highly oxygenated environment of living tissue results in chronic inflammation which produces universal inflammatory reaction by the host.

INTERACTION BETWEEN DEVICE AND MICROBE[23]:

Intravascular catheters become rapidly coated with serum constituents that facilitate the attachment organisms to foreign material. The microbial surface components recognize adhesive matrix molecules.

Adherence of microorganism to the catheter surface occurs with the help of

(1) Microbial factors such as fibrous glycocalyx

(2) Host factors such as fibronectin, fibrinogen, and laminin

(3) Physical character of catheter surface.

(4) Surface character of adherent bacteria

(5) Intrinsic phenotypic change of adherent bacteria.

MICRO ORGANISM ON CATHETER SURFACE TAKE TWO FORMS: [23,33,34]

Intrinsic phenotypic changes trigger the expression of several enzymes that catalyze the production of exopolysaccaride which in turn form biofilm. Biofilm producing bacteria are resistant to glycopeptide antibiotics and also resistant to the host defenses against such as phagocytes and antibodies. Bacteria in biofilm are metabolically active. Microbial colonization and biofilm formation on the catheter surface occurs within 24 hours of insertion.

a. **Sessile adherent forms** are those in which organisms are buried in the biofilm layer. They are retrieved by disruptive methods like VORTEXING.

b. **Planktonic free floating forms** in which the organisms disseminate over the catheter surface. They are retrieved by semiquantitative culture by ROLLPLATE METHOD.

a. Skin Insertion Site: :[26,29,52]

It is the common source of colonization and infection of vascular catheters occurring in < 10 days duration. Microbes migrate from the skin insertion site along the external surface and colonize distal intravascular tip of the catheter leading to blood stream infection. This was studied by Maki and diagnosed by Semiquantitative culture by roll plate method.

b. Hub Contamination:[23,29]

It is more common in catheter which is in place for > 30 days duration. Organisms are introduced into the hub from hands of the medical personnel. They migrate along the internal surface of catheter leading to luminal colonization and blood stream infection. This was studied by Sitges *et al* and diagnosed by Intraluminal quantitative culture.

Centers for Disease control (CDC) definition for central venous catheter – related blood stream infections[4].

Catheter exit – site infection

A positive semi-quantitative cultures of the drainage material in the presence of redness, crusting and exudates at the catheter exit site.

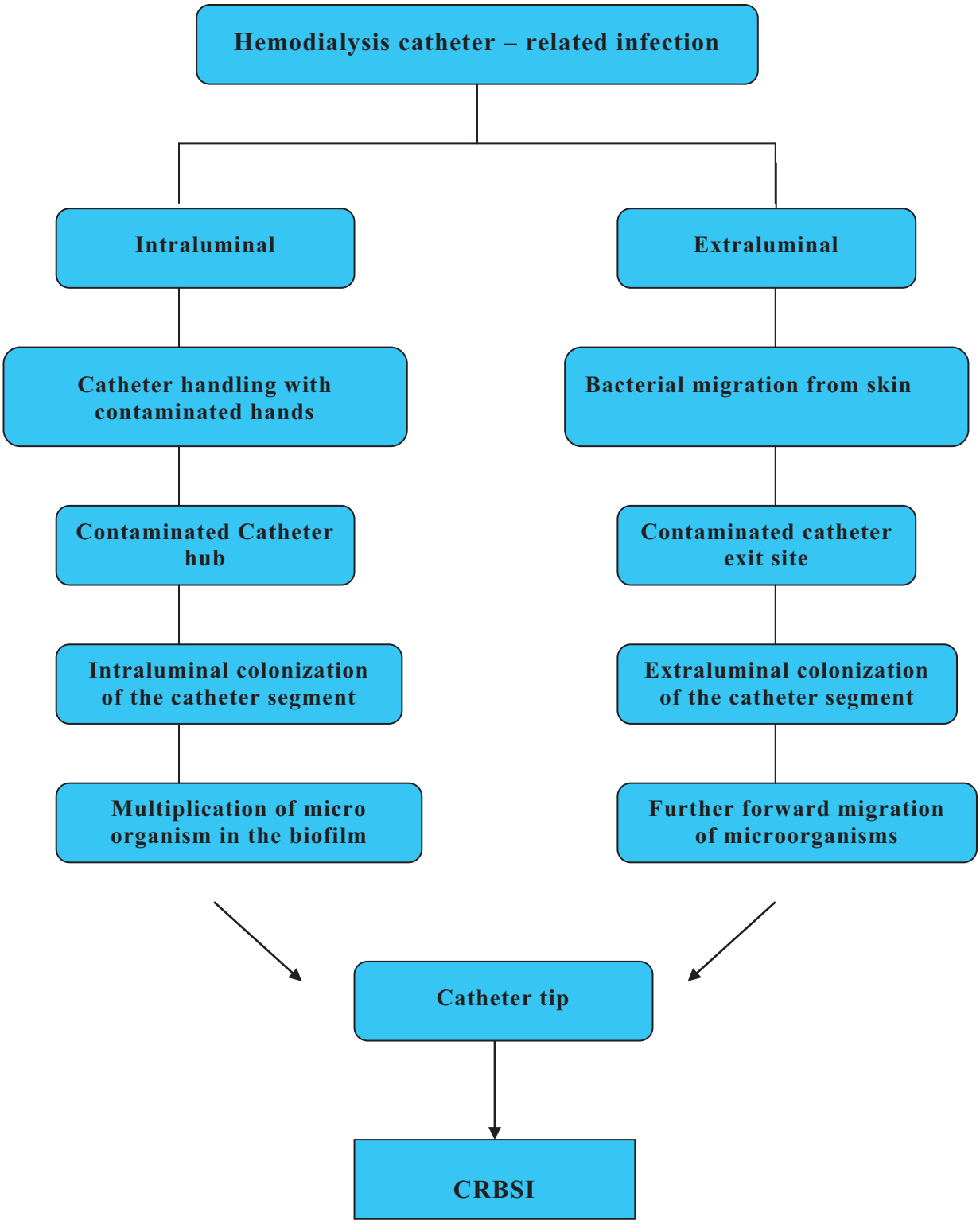
Catheter colonization

In the absence of clinical signs of infection at the catheter exit site, <10CFU (colony forming units) on quantitative culture (vortex method) or <15 CFU on semi quantitative cultures (roll-plate technique)

Catheter – related blood stream infection

The isolation of the same organism from a quantitative culture of the distal segment of the catheter and from the blood of a patient with clinical symptoms of sepsis in the absence of any other noticeable source of infection.

ORGANISMS CAUSING CATHETER RELATED BLOOD STREAM INFECTION ENTER THE BLOOD STREAM BY FOLLOWING METHODS



CLINICAL FEATURES OF CATHETER INFECTION:[7]

a. EXIT SITE INFECTION: Local warmth, Erythema. Tenderness, Purulent drainage, elevated temperature > 38 F. Exudate formation around 2 cms of the insertion site.

b. TUNNEL INFECTION: Resemble exit infection. Tenderness and erythema which extend up to chest along subcutaneous tract at proximal end of the catheter.

c. SEPTIC THROMBOPHLEBITIS: They reflect bacterial invasion and subsequent thrombosis of the vein. This is always bacteremic. Local signs of venous insufficiency such as edema of upper extremity are present.

d. BACTEREMIA: This occurs in the absence of localizing signs or symptoms and common in immuno compromised and neutropenic individuals. They may present with fever of unknown origin and leucocytosis within 2 days of catheterization.

IMMUNITY [23,46]

Renal failure patients are prone to suffer from impaired immune defence mechanisms. Cell mediated immunity is altered by the presence of uremia, nutritional abnormalities and inflammation induced by HD filters that cause oxidative stress, apoptosis and produce T cell depletion. MHC class II analogue protein expressed by Staphylococcus aureus may attenuate the host cell mediated immunity by T cell proliferative response to Gram positive bacterial infections. The degree of impairment is related to the duration of

uremic state, lymphopenia and decreased lymphocytic response to antigenic stimulation.

ANTIMICROBIAL RESISTANCE IN HEMODIOALYSIS

In an emergence of antimicrobial resistance, a susceptible bacteria undergoes mutation and become a new resistant bacteria. Methicillin resistant Staphylococcus aureus (MRSA) are increasingly reported due to frequent hospitalization and antibiotic exposure. ESRD patients are especially vulnerable to infections with MRSA. 13% of MRSA infection has been reported from United States between 2004-2006 and the mortality for CRBSI about 35%. MRSA accounts for more than 26.7% cases in India.[56] Beta lactamase producing organisms (ESBL) were reported about 6.3% in India

INDICATIONS FOR HEMODIALYSIS CATHETER REMOVAL[23]

Persistence of fever and positive blood culture while being on appropriate antibiotics for 36-48 hours.

Recurrence of fever and bacteremia despite adequate dosage and duration of systemic antibiotic administration

Exit site infections extending to catheter tunnel with severe sepsis

CRBSI associated with hypotension or signs of cerebral hypoperfusion.

Septic thrombosis of great veins as determined by a Doppler flow study.

Infective endocarditis and systemic septic embolisation.

OTHER ASSOCIATED INFECTIONS[47]

Wound infection: Pus or redness at the wound not related to the vascular access

Pneumonia: A new infiltrate or pneumia seen on chest radiograph

Urinary tract infection: Urine culture with >100,000 organism/ml with no more than two species isolated

DISINFECTION STRATEGIES [23,54]

Sterile technique and Hand hygiene:

Good hand hygiene before insertion of catheter, proper aseptic technique, full barrier precaution during insertion of catheter will reduce the incidence of catheter related infection

Cutaneous asepsis:

Povidone iodine, chlorhexidine, tincture iodine or 70% alcohol are used to clean the surface.

Catheter dressing:

Sterile, transparant, semipermeable dressings are used to cover the catheter. Antimicrobial / Antiseptic impregnated catheters : External surfaces of catheters are incorporated with Chorhexidine silver sulphadiazine

LABORATORY DIAGNOSIS

Standard blood culture methodology is the main stay of laboratory diagnosis. For the establishment of bacteremia, cultures should be drawn from distant site and not from the access device. Cultures drawn through the access site may help to document dialysis, may help in deciding whether to remove the catheter. The clinical appearance of the access site is important. Erythema, induration, edema and exudate confirm the presence of infection. Blood should be drawn in an aseptic manner with adequate volume and at an appropriate time before the usage of antibiotics.

A. CATHETER IN SITU:

ERBP recommends Catheter sparing strategies recently to avoid unnecessary and wasteful removal of catheters.

1. PAIRED QUANTITATIVE BLOOD CULTURE [48 – 51, 55]

Simultaneous blood sample from the catheter and peripheral site are taken. A ratio of catheter and peripheral blood of 4:1 is considered significant. It has several advantages for the following reasons such that it has an ability to detect the organism within the lumen of the catheter, to evaluate relative number of organisms from different segments of the catheter.

2. CATHETER SITE COLONIZATION [47,53]

Yield of single or predominant pathogen of culture of skin swab taken at the insertion site. Skin is the most common source for short term catheter colonization and infections.

B. CATHETER TIP PROCESSING

GRAM STAINING OF CATHETER TIP [8]

COOPER HOPKINS found Gram Stain to be highly diagnostic but reporting needs at least fifteen minutes of microscopic scanning of catheter surface.

DIRECT ACRIDINE ORANGE STAINING [8]

RUSHFORTH and ZUFFERY *et al* used direct acridine orange staining and reported high sensitivity and specificity. Disadvantage are labor intensive, high cost and low positive predictive value.

SEMIQUANTITATIVE ROLLPLATE METHOD [27,33,34]

This method was established by Maki *et al*. It retrieves organism from the external surface of the catheter. Roll plate of distal segment of the catheter and quantitative colony count indicates external surface colonization. It is significant if > 15 CFU detected.

VORTEXING [26,33,34]

It indicate internal and external surface colonization. The procedure involves vortexing of the catheter tip followed by quantitative culture on 5% Sheep Blood Agar. Michael Kelly *et al* has described that a colony count of >100 CFU/ml is significant.

BROTH CULTURE OF CATHETER TIP [1]

Catheter segment is immersed in trypticase soy broth, incubated at 37°C aerobically and examined daily for upto 72 Hours for turbidity.

MANAGEMENT [23,52,55]

It depends on the following principles:

1. Extent of infection whether local or systemic.
2. Micro organism causing infection
3. Underlying condition of the catheterized host
4. Intravenous treatment with appropriate antibiotics should be initiated on clinical grounds even without microbiological proof.
5. Treatment with antibiotics should be continued for prolonged period.

6. If an abscess is present at access site, it should be drained immediately.

7. If clinical improvement is not evident within 2 to 3 days, catheter should be removed.

EXIT SITE INFECTION

Treat with topical agents like povidone iodine, mupirocin. Sterile dressing and local care along with antibiotics without catheter removal will reduce the number of epispodes of bacteremia .

TUNNEL INFECTION:

More serious and requires catheter removal and appropriate antibiotics

CATHETER RELATED BACTEREMIA[23]

a. *Staphylococcus epidermidis* : Vancomycin - 7 days

b. *Staphylococcus aureus* : Semisynthetic pencillin - 2 weeks

c. Gram Negative Bacilli : Aminoglycosides - 1 week

d. *Candida* Species : Flucanozole - 14 days

IDSA recommendation of antibiotic dosing for patients who are undergoing hemodialysis :

Vancomycin 20mg/kg loading dose infused during last one hour of dialysis,500mg during the last 30 minutes of dialysis.

Ceftazidaime 1g IV after each hemodialysis.

Cefazolin 20 mg/kg after each hemodialysis

Candida infection

Caspofungin 70mg IV loading dose followed by 50mg IV daily.

Fluconazone 200mg orally daily.

PREVENTION

Following principles are useful in preventing infection: [52,55]

1) Hemodialysis unit is 'a restricted" site. Personnel and visitors should change and wear operating room type clothing and shoe covers.

2) All access site puncture should be performed with meticulous sterile technique. Disinfection of site with 3% Iodine followed by 70% Ethyl alcohol is preferred.

3) Regular cleaning schedule of dialysis unit with appropriate disinfectant should be established.

4) Dialysis supply system should be operated at near sterile condition.

5) Education of dialysis staff about design, operation, maintenance and potential hazards of Hemodialysis Equipment.

6) Establishment of experienced infusion therapy team to insert and maintain catheters.

7) Use of maximum sterile barriers.

8) Use of Tunneling and Ionic Silver Cuffs which act as antimicrobial deterrent and a physical barrier to migration of bacteria.

9) Flushing of tunelled catheters with Heparin and Intraluminal antibiotic Locks.

10) Antiseptic Hubs.

11) Antimicrobial Coating of Catheters.

12) Maximal sterile barrier precautions, asepsis and catheter dressing

13) Proper selection of site for catheter placement

14) Nasal decolonization of bacterial flora

AIMS & OBJECTIVES

1. To isolate and identify the bacteria and fungi from Central Venous Catheter related blood stream infections in patients undergoing hemodialysis

2. To screen the patients for other bacterial, fungal and parasitic infections

3. To study the risk factor associated with Central venous catheter related blood stream infection

4. To study the antibiotic and antifungal susceptibility pattern of the isolated bacterial and fungal organisms.

MATERIALS AND METHODS

In the Rajiv Gandhi Government General Hospital, Chennai, Hemodialysis was started in the year 1969, using Lucas Hemodialysis machine. Since then, hemodialysis facilities have improved. Now there are seven hemodialysis machines in the Hemodialysis unit of Department of Nephrology.

PLACE OF STUDY:

This cross sectional study was conducted in the Institute of Microbiology, in association with Department of Nephrology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai.

Study period and design

This cross sectional study was done for period of one year from September 2011 to October 2012.

Ethical consideration

Approval was obtained from the institutional ethical committee before the commencement of the study. Informed consent was obtained from the study population. All patients satisfying the inclusion criteria were documented. Patients were interviewed by structured questionnaire.

Study population

150 adult inpatients with acute and chronic renal failure who undergo hemodialysis through central venous catheter, suspected having infection based on the clinical symptoms and signs were taken for the study.

CASE DEFINITION [4,34,52,53,56,57]

Catheter Colonization

The yield of $\geq$ 15 CFU from a catheter, by means of semiquantitative culture or a yield of $\geq 10^2$ from a catheter, by means of quantitative culture in the absence of signs of infection is considered indicative of catheter colonization.

Catheter related infection

The yield of $\geq$ 15cfu from a catheter by means of semiquantitative culture or a yield of $\geq 10^2$ from a catheter, by means of quantitative culture with accompanying signs of local or systemic infection, is indicative of CRI.

Catheter related blood stream infection:

The isolation of the same organism from a quantitative culture of the distal segment of the catheter and from the blood of a patient with clinical symptoms of sepsis in the absence of any other noticeable source of infection.

Inclusion Criteria

a) Adult inpatients undergoing hemodialysis, who develop signs of inflammation at different sites like Jugular, Femoral and Subclavian catheterization after 48 hrs of insertion of catheter.

b) Patients who develop fever, chills, headache, abdominal pain, diarrhea and hypotension and any other signs and symptoms suggestive of infection any time after 48hrs of insertion of central venous catheter during hospitalization.

EXCLUSION CRITERIA

a) Patients who have fever, chills, head ache and signs of inflammation within 48 hrs of insertion of catheter or prior to dialysis

b) Patients in whom blood culture was positive before dialysis

c) Patients with AV fistula or AV graft

DATA COLLECTION

Data collection included name, age, address, date of admission, diagnosis at admission, physical examination finding. Duration of hospital stay, nutritional status, underlying illness like diabetes mellitus, uremia, hypertension, history of previous dialysis, infection during last dialysis, blood transfusion, type of catheter, duration of catheterization and concurrent other infections were recorded.

STATISTICAL ANALYSIS

Statistical analyses were carried out using Statistical Package for Social Sciences (SPSS) and Epi-Info softwares by a statistician. The proportional data of this cross sectional study were tested using Pearson's Chi Square analysis test, Fisher exact probability test and Binomial proportion test.

SAMPLE COLLECTION AND PROCESSING

The following samples were collected

- Catheter tip
- Peripheral venous blood
- Catheter blood (catheter in situ)
- Swab from infected site of catheter
- Stool
- Urine
- Sputum

CATHETER TIP[57]

Catheter Removal Technique

The skin exit site was inspected for evidence of local infection. Area was cleaned with 70 % isopropyl alcohol and allowed to dry. The catheter was then clamped with sterile Spencer well Forceps as close to skin as possible.

The catheter was sectioned distally with sterile blade and carefully removed avoiding skin contact and placed in a sterile test tube and transported to the laboratory. The catheter was cut into three segments and used for quantitative and semiquantitative culture.

One segment of the catheter was immersed in trypticase soy broth and another segment was used for roll plate method. The third segment was used for endoluminal washing method.

Gram Staining of Impression Smear of Catheter Tip[2,27,53,98]

A shallow narrow line of sterile saline solution was made along centre of long axis of glass slide with pasteur pipette. The catheter was removed from transport container by inserting microbiological straight wire into lumen. The external surface of catheter segment was rotated on the spot in line of saline solution by rotating handle of straight wire between index finger and thumb and attempting to keep the saline solution as narrow as possible. A zigzag manner along the long axis of slide was also used to dislodge as much as material as possible from outside of catheter. Slides were air dried, heat fixed and stained by Gram Stain method and examined by conventional light microscopy under oil immersion.

Semi quantitative Culture of Catheter [2]

Roll plate method [2,27,52,]

First segment of the catheter 2 cm long was transferred on to the surface of a 5 % sheep blood agar and using sterile forceps or swab. The catheter segment was rolled across the plate back and forth about four times and incubated at 37^0C for 48hrs.

Colonies on the plate were enumerated, identified and expressed in colony forming units (CFU). A colony count of 15 colonies or greater was considered as positive culture and less than 15 colonies was taken as contamination. These colonies were identified using various biochemical reactions.

Interpretation

12-24 hrs- If no growth after 24 hours the culture was reported as NO GROWTH. If >15 colonies suggested as local catheter infection. If < 15 colonies not infection and suggestive of contaminated. Declare the result with antibiotic susceptibility after 48 hours.

Vortex Method [2,23,26],

In a sterile test tube middle segment of the catheter was placed inside the trypticase soy broth and vortexed in a vortex shaker machine for 1 minute. 0.1 ml of the suspension was streaked onto sheep blood agar plate and incubated at 37^0C. If no growth on the plate was evident, the broth was observed for turbidity and subcultured every 72 hrs upto 10 days. If turbidity, or haemolysis was noted in the bottles, subcultures were done into Blood Agar, MacConkey Agar and Sabouraud's Dextrose Agar Slope.

Interpretation

> 100 CFU/ml for catheter segment was considered as significant.

Segment washing (or) Endoluminal Flush Technique[56]

1 ml of sterile saline was flushed into the lumen of the catheter segment using a sterile syringe into a sterile vial. 0.01ml of suspension was streaked on to 5% sheep blood agar and MacConkey agar and incubated at 37^0C in CO_2 for quantification. >100 CFU/ml for catheter segment was considered as significant

COLLECTION OF BLOOD

Blood was drawn before the use of antibiotics. The venipuncture site was prepared with soap and water. Rinsed with sterile water, applied 1-2% tincture of iodine or povidone iodine or 70% isopropyl alcohol which will kill the surface and subsurface bacteria. The surface the skin was cleaned in a concentric manner and allowed to dry. 21ml of blood was drawn under aseptic precaution.

BLOOD FROM CATHETER: (CATHETER IN SITU)

Qualitative Culture of Catheter Blood

Under aseptic precaution The Luer lock of the catheter was removed, the first 5m1 of the aspirated blood from each catheter was discarded and then 12m1 of blood was aspirated of which 10 ml was inoculated aseptically into BHI broth and 2ml of blood was added into trypticase soy broth and incubated under standard conditions.

Quantitative Culture of Catheter Blood

One ml of sterile blood was collected aseptically with syringe from the catheter and mixed with 19 ml of melted nutrient agar base at 46°C and poured into sterile 9 cms petridish. The plates were rotated to distribute blood uniformly and left to solidify and incubating aerobically at 37°C for 48 hours. The colony count of > 100 colonies / ml was considered significant.

BLOOD FROM PERIPHERAL VEIN

1. Qualitative Culture of Peripheral Blood [2,23]

The site for venipuncture identified and tourniquet was applied. The skin was prepared exclusively with 70 % isopropyl alcohol then swabbed concentrically and 21 ml blood was collected using a sterile disposable syringe and needle.

10 ml was added aseptically into brain heart infusion broth (BHI) and 10 ml was added into trypticase soy broth. The remaining 1 ml of blood was used for quantitative culture. After collection the ratio of blood to medium should be 1:10. The contents are mixed well for uniform distribution. The BHI broth and trypticase soy broth were incubated at 37°C aerobically and examined daily for a period of ten days. If turbidity, or haemolysis was observed in the bottles, subcultures were done into Blood Agar, MacConkey Agar and Sabouraud's Dextrose Agar Slope. The plates were incubated at 37°C aerobically for 48 hrs and observed for growth.

Quantitative Culture of Peripheral venous Blood[2]

One ml of sterile blood which was collected aseptically was added to 9cms sterile petridish which contains l9ml of previously melted Nutrient agar

base at 46°C. The plates were rotated to distribute blood uniformly and left to solidify before incubating it aerobically at 37° C for 48 hours. Once colonies were seen, they were counted accordingly and when the count was greater than 100cfu/ml it was considered significant.

Collection of Swab [3]

Any purulent discharge, serous or serosanguinous discharge from the catheter site has been swabbed by using sterile swab. Two swabs were collected in a sterile test tubes and transported immediately to the laboratary. One swab was subjected to Gram stain and another for culture on 5% sheep blood agar and MacConkey agar.

Collection of stool [59]

Sterile wide mouth container with spatula was used for collection of liquid and semisolid stool and transported immediately and examined for wet mount, iodine mount and modified acid fast staining using 1% sulfuric acid. Enriched with Selenite F broth also plated directly on MacConkey agar. If growth of non lactose fermenting colonies observered it would be further subcultured on Xylose lysine deoxycholate agar and Deoxycholate citrate agar.

Collection of Urine [60]

Clean catch midstream urine was collected in a sterile container after proper instruction and transported to the laboratory immediately. Direct Grams staining and Quantitative culture on MacConkey and 5% Sheep blood agar was done. A colony count of $>10^5$ was considered significant.

Collection of sputum:[60]

After through washing of mouth with water well expectorated early morning sputum sputum was collected in a wide mouth sterile container and transported immediately the laboratory. Sputum was treated with equal volume of 4% sodium hydroxide. Direct Grams staining, 10% Pottosium hydroxide mount , Ziehl Neelsen staining, Modified acid fast staining was done.

PROCESSING OF SPECIMEN

Gram Staining Procedure

Thin smear of the specimen was prepared on a clean sterile glass slide. Then the smear was fixed by heating over a bunsen burner flame. The smear was flooded with 1% methyl violet for 1 minute and washed with distilled water. The smear was flooded with gram's iodine for 1 minute and washed with distilled water and decolorized with acetone, washed with distilled water and counter stained with dilute carbol fuschin for 30 seconds

Wet Mount

In a clean glass slide about 2 mg of faeces was mixed with a drop of saline, cover with a coverslip and examined under low and high power for trophozoites and cysts.

Iodine mount

In a clean glass slide small quantity about 2mg of stool was mixed with a drop of Lugol's iodine, cover with a coverslip and examined under low and high power for eggs.

KOH Wet Mount

A clean glass slide was taken. The specimen was placed in the centre of the slide. A drop of 10% KOH was added and a coverslip was placed over that, observed under low and high power microscope.

Peripheral smear

Tip of the finger was cleaned with 70% alcohol and allowed to dry. One drop of capillary blood was drawn by finger prick using sterile needle and thin smear was prepared.

Giemsa Staining

Air dry and fix the smear with absolute methanol for 2-3 minutes. Stain the slide with Giemsa stain for 30 minutes. Air dry the slide and observe under the oil immersion microscope.

Ziehl Neelsen staining

Smear was prepared on a clean glass slide and allowed to dry in the air. The smear was heat fixed over a Bunsen burner flame. The smear was flooded with strong carbol fuschin stain for 5 - 8 minutes with intermittent heating without boiling. The smear decolourized with 20% sulfuric acid and counterstained with loeffler's methylene blue for 1-2 minutes.

Modified Acid Fast Staining

Thin smear of the specimen was prepared and dried in the air. The smear was fixed by heating over a Bunsen burner flame. The smear was flooded with strong carbol fuschin stain for 5 minutes. Washed with distilled water and flooded with 1% sulphuric acid for 3 minutes. Washed with

distilled water and counter stained with 3% methylene blue for 3 minutes. Washed with distilled water dried and examined under oil immersion microscope.

CULTURE

The samples were plated onto the following media. 5% sheep blood agar, chocolate agar, Mac conkey agar, All the inoculated plates were incubated at 37^0C under aerobic condition and in a carbondioxide enriched atmosphere. Plates were evaluated for growth at 24 and 48 hours and discarded after five days except sabouraud dextrose agar which was kept for 4 weeks.

INTERPRETATION OF BACTERIAL CULTURE

Interpretation isolates were identified by means of colony morphology, Gram staining, motility and biochemical reactions by standard microbiological techniques as recommended by clinical and laboratory standards institute (CLSI)

After 24 hours of incubation, identification of bacteria was done by studying morphology of colony, gram stain, motility, catalase and oxidase tests. Single colony was taken and processed along with the controls.

- Gram staining
- Hanging drop
- Oxidase test
- Catalase test

- Coagulase test
- Phosphatase test
- IMVIC test
- Nitrate reduction test
- Urease test
- TSI (Triple sugar iron agar)
- Phenyl alanine deaminase test
- O-F test
- Sugar fermentation test
- LAO decarboxylases test

OXIDASE TEST

1% tetramethyl - p - phenylene diamine dihydrochloride was prepared freshly with sterile distilled water. A filter paper circle was placed into a sterile petridish and moistened with several drops of the fresh reagent. A colony from a nutrient agar was taken with a sterile glass rod and rubbed onto the moistened filter paper along with controls. Appearance of dark purple colour within 10 seconds was considered as positive.

CATALASE TEST

Single colony from nutrient agar plate was picked with a sterile glass rod and inserted into 1ml of 3% hydrogen peroxide solution in a small clean

test tube. Immediate and sustained production of gas bubbles from the colony indicate positive reaction.

COAGULASE TEST

This was done to detect both free and bound coagulase enzymes.

Slide coagulase test:

Slide test is a rapid test to detect bound coagulase. Two drops of normal saline were placed in two circles drawn on a glass slide. Growth was taken from nutrient agar plate and emulsified into smooth suspension in two circles. Then one drop of undiluted plasma was added to one circle which was marked as test and the other circle as control without plasma. Visible clumping within 10 - 15 sec of mixing the plasma with the suspension was taken as positive.

Tube coagulase test:

This test detects free coagulase. A small amount of the colony growth of the organism is emulsified in a tube containing 0.5ml of coagulase plasma. Incubate the tube at 35^0C for 4 hours and observe for clot formation by gently tilting the tube. If no clot is observed at that time, reincubate the tube at room temperature and read again after 18 hours.

INDOLE TEST

Organisms were inoculated into Tryptophan broth and incubated a 37°C for 18-24 hrs. 15 drops of Kovac's reagent was added along the inner wall of the tube. Appearance of red ring over the surface was taken as positive.

METHYL RED TEST

Organisms were inoculated into Glucose Phosphate broth and incubated at 37°C for 48-72hrs. Then 5 drops of Methyl red reagent was added to the broth. Appearance of red colour indicated positive result.

VOGES-PROSKAUER TEST

Organisms were inoculated into Glucose Phosphate broth and incubated at 37°C for 48-72 hrs. Then 0.6m1 of 5% alpha naphthol was added, followed by 0.2ml of 40% KOH. The tube was gently shaken without cotton plug to expose the medium to atmospheric oxygen and was read after 10-15 minutes. Development of red color within 15 minutes was taken as positive.

CITRATE UTILIZATION TEST

A well isolated colony is picked from the surface of a primary isolation medium and inoculated as a single streak on the slant surface of the citrate agar tube. The tube is incubated at 35^0C for 24 to 48 hours. The test is positive if there is development of a deep blue colour within 24 to 48 hours, indicating that the test organism has been able to utilize the citrate contained in the medium.

NITRATE REDUCTION TEST

Nitrate broth was inoculated with test organisms and incubated at 37°C for 24 - 48 hrs. 5 drops of each reagent A(α naphthylamine) and reagent B (sulfanilic acid) were added to the broth. Red color developed within few minutes indicating the presence of nitrite .

UREASE TEST

The entire slope of the Christensen's medium was inoculated with test organisms and incubated at 37°C for 24 - 96 hrs. Urease producing organisms changed the colour of medium to purple pink.

SUGAR FERMENTATION TESTS

Sugar fermentation test media containing different sugars in the concentration of 1%, with inverted Durham's tubes were inoculated with test organisms, and incubated at 37°C and observed for up to 1 week. Change of colour to yellow indicated that the sugar was fermented. Presence of gas in Durham's tubes indicated gas production.

O-F TEST

Two tubes of Hugh - Leifsons test media were stab inoculated with test organisms. One tube of this pair was covered with a 1cm layer of liquid paraffin and the other tube was left open to air. Both tubes were incubated at 37°C and examined daily for up to 7 days. Appearance of yellow color in open tube and green color in covered tube indicated oxidative utilization of the sugar.

TRIPLE SUGAR IRON TEST

The organism was stabbed into butt and streaked onto the surface of slant, incubated at 37°C overnight. The next day change of color, H_2S production and presence of gas were noted.

PHENYL ALANINE DEAMINASE TEST

The medium containing phenyl alanine deaminase was inoculated heavily with test organism, incubated at 37°C overnight. Next day few drops

of 10% solution of ferric chloride was allowed to run down over the growth. Appearance of green color in the fluid and in the slope indicated positive test.

PHOSPHATASE TEST

Organism was grown on phenolphthalein diphosphate agar (consist of l000ml of nutrient agar and l0ml of 1% aqueous solution of sodium phenolphthalein diphosphate) at 37°C overnight. Next day few drops of liquor ammonia was poured on lid and plate was inverted over the lid. Colonies turned to bright pink, from yellow color within a few minutes, due to liberation of free phenolphthalein by the action of phosphatase was considered as positive.

LAO DECARBOXYLASES TEST

A well isolated colony of the test organism was inoculated into two tubes of Moeller decarboxylase medium, one containing the aminoacid to be tested, the other was used as control devoid of aminoacid. The tubes were overlaid with sterile liquid paraffin and incubated at 35^0c for 18 to 24 hours. Conversion of the control tube to yellow colour and reversion of the tube containing the aminoacid to a blue purple colour indicated positive test. The specific amine products were

Lysine – cadaverine

Ornithine – putrescine

Arginine - citrulline

INTERPRETATION OF FUNGAL CULTURE

Inoculated SDA slants were incubated at 25^0C and 35^0C for minimum of 4 weeks before discarding as negative. These slants were inspected daily during the first week and twice weekly during the next three weeks for growth.

Identification of filamentous fungi was done by preparing Lacto Phenol Cotton Blue mount and studying the morphology of hyphae and conidial arrangement. In difficult ambiguous cases where sporulation was inadequate, Riddle's slide culture technique was performed.

In case of yeasts, identification and specification was done by Gram's stain morphology, germ tube test, Chrome agar and biochemical tests by standard microbiological techniques as recommended by CLSI.

Examination of Sabouraud's dextrose agar Media:

The colonies were observed for growth in the Sabouraud's dextrose agar slopes and noted the description, if it was inadequate reincubated and examined daily during first week and twice a week for next 3 weeks. Failure of growth after 6 weeks was considered as negative for fungal growth and is to be discarded.

Germ tube test (Reynolds-Braude Phenomenon)

Suspension of test organism in 0.5ml of sterile serum (plasma, egg albumin or peptone media) and incubated at 37^oC for 2 hours. A drop from incubated serum placed on the slide with coverslip and observed under microscope for germ tubes

Lactophenol cotton blue stain

The fungal growth was taken from Sabouraud's dextrose agar slope with spud and transferred onto the clean glass slide and two to three drops of Lactophenol cotton blue reagent was added over the fungal growth. By using teasing needles the growth was spread over the slide and coverslip was placed without trapping any air bubbles. The morphology of hyphae, conidia were observed under microscope and was correlated with macroscopic features.

Chrom Agar

The species of *candida* can be identified by different coloured colonies produced due to the reaction between specific enzymes of the different species and the chromogenic substrates in the system, which are as follows:

- *C.albicans* - light green
- *C.dubliniensis* - dark green
- *C.glabrata* - pink to purple
- *C.krusei* - pink
- *C.parapsilosis* - cream to pale pink
- *C.tropicalis* - blue with pink halo

Sugar Fermentation:

Biochemical tests like sugar fermentation was done for identification of yeast isolate Glucose, Maltose, sucrose, Lactose, Galactose and Trehalose sugars (2%) were used. The colour change in the tube containing the particular sugar indicates the yeast's ability to ferment carbohydrate.

Slide culture

The slide culture was performed using isolates. The slide culture is used to study undisturbed morphology details particularly relationship between reproductive structure like conidia, conidiophores and hyphae. Fungal slide culture was performed in cases with doubtful morphology.

ANTIMICROBIAL SENSITIVITY TESTING:

Antibiotic susceptibility testing was performed by the Kirby Bauer disc diffusion method on Mueller Hinton agar according to Clinical Laboratory Standards Institute protocols. The diameters of zones of inhibition were interpreted according to CLSI standards for each organism. Culture media and antibiotic discs were tested for quality control using standard ATCC strains.

The following standard strains were used

1. Staphylococcus aureus-ATCC 25923

2. Escherichia coli-ATCC 25922

3. Pseudomonas aeruginosa-ATCC 27853

The panel of antibiotics included in the antimicrobial sensitivity testing for

GNB

Ciprofloxacin, Cefotaxime, Amikacin, Garamycin, Ofloxacin, Norflox, Nitrofurantoin, Cotrimoxazole, Imipenam, Piprazine Tazobactum, Ceftazidime.

GPC

Cirofloxacin, Cephalexin, Amikacin, Erythromycin, Cotrimoxazole, Oxacillin, Penicillin, Amoxycillin, Cephelexin, Ciprofloxacin. Vancomycin, Amoxycillin clavulinic acid

PROCEDURE OF KIRBY-BAUER DISC DIFFUSION TEST

1) With a wire or a loop touched the surface of 5 similarly appearing colonies on an agar plate culture. Transferred the growth to a tube containing a suitable broth medium.

2) Allowed the culture to incubate at 35^0C until it matches the turbidity of 0.5% Mc Farland standard.

3) Dipped a sterile non-toxic cotton swab into the inoculum suspension and rotated the swab several times with firm pressure on the inside wall of the tube to remove excess of fluid.

4) Inoculated the dried surface of a Mueller Hinton agar plate that has been brought to room temperature by streaking the swab 3 times over the entire agar surface rotating the plate approximately 60 degrees to ensure an even distribution. Replaced the lid of the dish. Allowed 3 to 5 minutes but no longer than 15 minutes for the surface of the agar to dry before adding the antibiotic discs.

5) Appropriate antimicrobial disc was placed on the surface of the agar using forceps.

6) Plate was incubated at 37^0 c overnight.

7) After overnight incubation, zone diameters was measured in mm from the edge of the disc to the zone edge with a ruled template on the agar surface

Zone diameters were interpreted according to CLSI guidelines

For gram negative bacteria, the following antibiotics were included in the antimicrobial sensitivity testing (Himedia)

ANTIMICROBIAL AGENT	DISC CONTENT	ZONE DIAMETER IN mm		
		S	I	R
Amikacin	30 µg	≥17	15-16	≤14
Gentamycin	10µg	≥15	13-14	≤12
Cefotaxime	30µg	≥26	23-25	≤22
Ceftriaxone	30 µg	≥23	20-22	≤19
Ceftazidime	30µg	≥21	18-20	≤17
Ciprofloxacin	5 µg	≥21	16-20	≤15
Ofloxacin	5 µg	≥16	13-15	≤12
Imipenem	10µg	≥23	20-22	≤19
Norfloxacin	10 µg	≥17	13-16	≤12
Nitrofurantoin	300 µg	≥17	15-16	≤14
Piperacillin-tazobactam	100/10 µg	≥18	-	≤17

For Pseudomonas

ANTIMICROBIAL AGENT	DISC CONTENT	ZONE DIAMETER IN mm		
		S	I	R
Ceftazidime	30µg	≥18	15-17	≤14
Imipenem	10µg	≥16	14-15	≤13
Piperacillin-tazobactam	100/10 µg	≥18	-	≤17

For gram positive cocci, the following antibiotics were included in the antimicrobial sensitivity testing (Himedia)

ANTIMICROBIAL AGENT	DISC CONTENT	ZONE DIAMETER IN mm		
		S	I	R
Amikacin	30 µg	≥17	15-16	≤14
Amoxicillin-clavulanic acid	20/10 µg	≥18	14-17	≤13
Oxacillin	1 µg	≥13	11-12	≤10
Cefoxitin	30 µg	≥22	-	≤21
Ciprofloxacin	5µg	15	16-20	21
Ofloxacin	5 µg	≥18	15-17	≤14
Erythromycin	15 µg	≥23		≤13
Cotrimoxazole	1.25-23.75µg	≥10	11-15	≥16

DETECTION OF BETA LACTAMASE ENZYME PRODUCTION IN GRAM NEGATIVE BACILLI:

A) Extended Spectrum Beta Lactamases (ESBL) Detection Methods:

1. Screening method:[61]

Isolates of gram negative bacilli showing the following resistance pattern were considered to be possible ESBL producing strains.

Antibiotic	Zone diameter for possible ESBL producing strain
Ceftazidime (30µg)	<=22mm
Cefotaxime (30µg)	<=27mm
Ceftriaxone (30µg)	<=25mm
Aztreonam (30µg)	<=27mm

CLSI phenotypic confirmation test:[62,63]

With a sterile bacterial loop 3-5 identical colonies were picked from a fresh overnight culture and inoculated into 5 ml of nutrient broth. The broth was incubated at 35°C for 2-4 hrs and the turbidity matched with 0.5 McFarlands standard. Lawn culture of the test organism was made on to Muller-Hinton Agar (MHA) plate. Antibiotic disc Ceftazidime (CAZ 30µg) and Ceftazidime/Clavulanic acid (CAZ/ CA 30µg/ l0µg) were placed and incubated at 35°C overnight. A >5mm increase in zone diameter for Ceftazidime tested in combination with Clavulanic acid versus its zone when tested alone confirmed an ESBL producing organism.

Double disk diffusion synergy test:[64]

In this test discs of third generation Cephalosporins and Clavulinic acid (20µg/l0µg) were kept 30mm apart from centre to centre on inoculated Muller-Hinton Agar. A clear extension of the edge of the inhibition zone of cephalosporin towards Clavulinic acid disc was interpreted as positive for ESBL production

ESBL DETECTION BY E- TEST STRIP

This combines both the principles of dilution and diffusion techniques. E strip is a thin non porous plastic strip 5mm wide and 60mm long. It carries two shorter gradients aligned in opposing directions on a single strip. One end generates a stable concentration gradient of the one of the oxyimino cephalosporins (eg. ceftazidime), while the other end generates a gradient of cephalosporin + clavulanic acid (4µg/ml). When applied to an inoculated agar plate inhibition ellipse may be seen on the both ends of the strip. MIC is interpreted as the point of intersection of the inhibition ellipse with E test strip edge. Ratio of cephalosporin MIC and cephalosporin clavulanic acid MIC $\geq$8 indicates positive result.

AMPC β LACTAMASES DETECTION METHODS:

1. Screening method:[65]

A 0.5 Mcfarland of the test isolate was swabbed on MHA plate and disc of Cefotaxime (30µg), Ceftazidime (30µg) were placed adjacent to Cefoxitin (30µg) disc at a distance of 20 mm from each other. After incubation, isolates showing blunting of Ceftazidime or Cefotaxime zone of

inhibition adjacent to Cefoxitin disc or showing reduced susceptibility to either of the above drugs and Cefoxitin were considered as screen positive and selected for detection of AmpC β lactamases

2. AmpC Disc test:[65]

A lawn culture of E.coli ATCC 25922 were prepared on MHA plate. Sterile discs (6 mm) were moistened with sterile saline (20µl) and inoculated with several colonies of test organism. The inoculated disc was then placed beside a Cefoxitin disc (almost touching) on the inoculated plate. The plates were incubated overnight at 35°C. A positive test appeared as a flattening or indentation of the Cefoxitin inhibition zone in the vicinity of the test disc. A negative test had an undistorted zone,

METALLO BETE LACTAMASES (MBL) DETECTION METHODS:

Imipenem- EDTA disc method:[66]

A l0µg imipenem disc containing 750 µg of EDTA solution was used. The inhibition zone with imipenem-EDTA disc were <19 mm for the MBL negative isolates and >23 mm for MBL positive isolates.

Imipenem- EDTA combined disk test:[67]

The test organism was inoculated onto MHA plate. Imipenem (10µg) and l0µg Imipenem disc containing 750pg of EDTA solution were placed on the plate and incubated overnight. If the increase in inhibition zone with Imipenem- EDTA disc is >7mm than the Imipenem disc alone, it was considered MBL positive.

DETECTION OF METHICILLIN RESISTANCE IN STAPHYLOCOCCUS AUREUS:

Disc diffusion method:[68,69]

Colonies isolated from agar culture plate were suspended directly into broth, vortexed to reach 0.5Mcfarlands standard. A lawn culture of the Staphylococcal colonies was made on the MHA plate and Cefoxitin 30µg disc was applied. Incubated was at 35^0C for 24 hours in ambient air. According to CLSI criteria with 30µg cefoxitin disc diameter of <21 or >22mm corresponded to resistant or susceptible to cefoxitin.

Minimum inhibitory concentration (MIC) for detecting Vancomycin resistance:[70]

1. Culture media: cation adjusted Mueller Hinton broth. (pH 7.2-7.4)

2. Preparation of stock antibiotic solution:

   Antibiotic stock solution can be prepared using the formula

   = 1000/p x VxC=w

   Where p= potency of the antibiotic in relation to the base. (For vancomycin, p 950/1000 mg; Himedia)

   V = volume of the stock solution to be prepared (l0ml)

   C = final concentration of the antibiotic solution (1024µg/ml)

   W = weight of the antibiotic to be dissolved in the volume V

Scheme of preparing dilution of antibiotics

- Arrange two rows of sterile test tubes in the rack (1 row for the test & 2nd for ATCC control)

Using sterile syringe transfer 2m1 of MH broth to the sterile container containing the working stock solution (128µg/mI concentration) from this transfer 1 ml to the first tube in each row.

- Now we have 2m1 of the diluted antibiotic in the sterile container. Using syringe add 2ml of MH broth to the 2m1 of left over antibiotic in the sterile container, mix and transfer 1 ml to second tube in each row.
- Repeat this procedure till the 8th tube
- Place 1 ml of the antibiotic free broth in the last tube in each row (growth control)
- The sterility controls for the antibiotic solution is kept

4. Inoculum preparation for the test and ATCC control and incubation

- Take 9.9 ml of MH broth in a sterile container.
- Add 0.1 ml of 0.5 Mcfarland turbidity matched test organism broth.
- Mix well, transfer 1 ml of inoculum using 2 ml syringe to each tube containing antibiotic dilutions and also to the control tube.
- Similarly repeat the procedure for ATCC control strain.
- Incubate the rack at 37°C – overnight.

- Observe the MIC of ATCC control strain, If it is out of the sensitive range, the test is invalid.

- Read for the test organism

- The lowest concentration of the antibiotic in which there is no visible growth will be the MIC for the drug & for the test organism

- MIC <2mg/ml – SENSITIVE- *Staphylococcus aureus*

ANTIFUNGAL SUSCEPTIBILITY TESTS

As per the guidelines of CLSI, the test was performed. MIC of water soluble drug Fluconazole and water insoluble drugs Amphotericin-B, Itraconazole and Voriconazole were determined.

BROTH DILUTION METHOD

Preparation of stock solution

Weight (mg) = volume (ml) x concentration (µg/ml)/Assay Potency

Incolum preparation

The isolateion were subcultured on SDA, fresh culture of 24 hours colonies were taken 0.5 Mc Farland standard inoculum was prepared and diluted upto 1:20 with normal saline. The final inoculum was prepared. The Suspension was mixed for 15 minutes for turbidity adjusted ether visually or with a spectro photometer by adding sufficient sterile saline (or) adding more colonies and to match with 0.5 Mc Farland at 530 nm wave length with the optical density of 0.09 to 0.11.

Stock solution for water soluble drugs- 5120 µg/ml

Stock solution for water insoluble drugs – 1600 µg/ml

Media used- RPMI 1640. Varying concentrations of the drugs were tested.

Amphotericin B	0.0313 to 16µg/ml
Fluconazole	0.125 to 64µg/ml
Itraconazole and Voriconazole	0.0313 to 16µg/ml

Preparation

Dimethyl sulfoxide (DMSO) was used to dissolve Amphotercin B, Itraconazole and voriconazole. The DMSO, the series of dilution at 100 times the final concentration were prepared from the Antifungal stock solution.

ATCC *Candida albicans* 90028 and ATCC *Aspergillus flavus* 204304 was used for quality control of the test. The broth microdilution test was performed by using sterile, disposable, multiwell microdilution plates (96U-shaped wells). 100µl of varying drug concentrations was dispensed in each rows from 1 to 10. 100µl of inoculum was also dispensed. Incubated at 35^0C for 48 hours.

INTERPRETATION:

Minimum inhibitory concentration was as defined as the lowest drug concentration that showed 100% inhibition of the growth compared to growth control well.

The test was read in comparison with growth control and antibiotic control. The adequate inhibition (or) clearance of growth after 24-48 hours was taken as MIC.

RESULTS

TABLE 1: DISTRIBUTION OF AGE & SEX IN THE STUDY GROUP (n=150)

Age in yrs	Male n =78 (52%)		Female n =72 (48%)		Total no (%)
	No.	*%*	*No.*	*%*	
18-20	2	2.6	4	5.6	6 (8.2%)
21-30	32	41.0	19	26.4	51 (67.4%)
31-40	19	24.4	11	15.3	30 (39.0%)
41-50	15	19.2	25	34.7	40 (53.9%)
51-60	8	10.2	7	9.7	15 (19.9%)
61-65	2	2.6	6	8.3	8 (8.9%)

78 males and 72 females were included in the study. Majority of the patients (67.4%) in the study group belonged to the age group of 21-30 years and highest percentage of cases (41%) seen in males of age group between 21-30 and in females(34.7%) seen in 41- 50 yrs of age group.

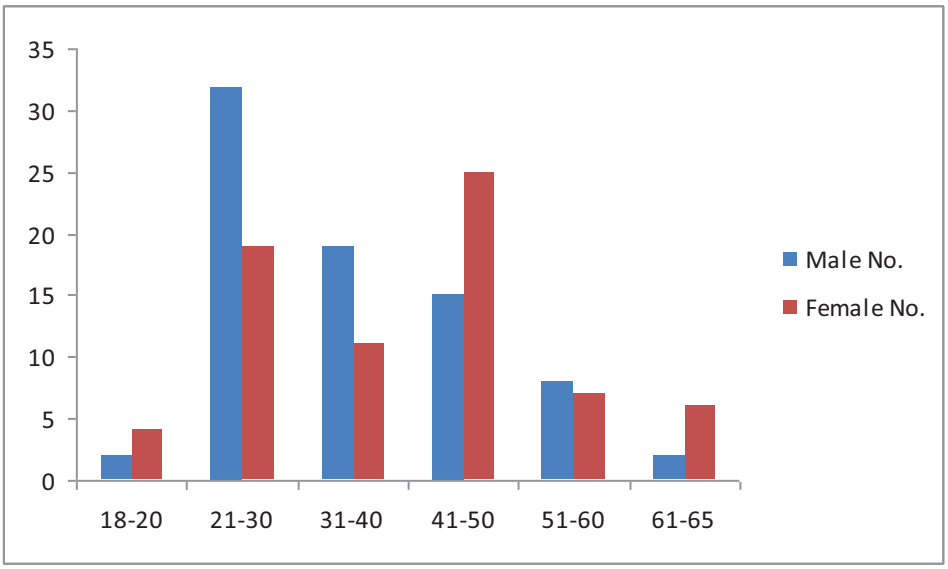


Fig1: Distribution of Age and Sex

TABLE 2: TYPE OF RENAL DISEASE IN THE STUDY GROUP (n=150)

Diagnosis	No.	%
Chronic Kidney Disease(CKD)	106	70.7
Acute Kidney Injury(AKI)	44	29.3

Majority of cases undergoing hemodialysis had CKD 70.7%, followed by AKI 29.3%

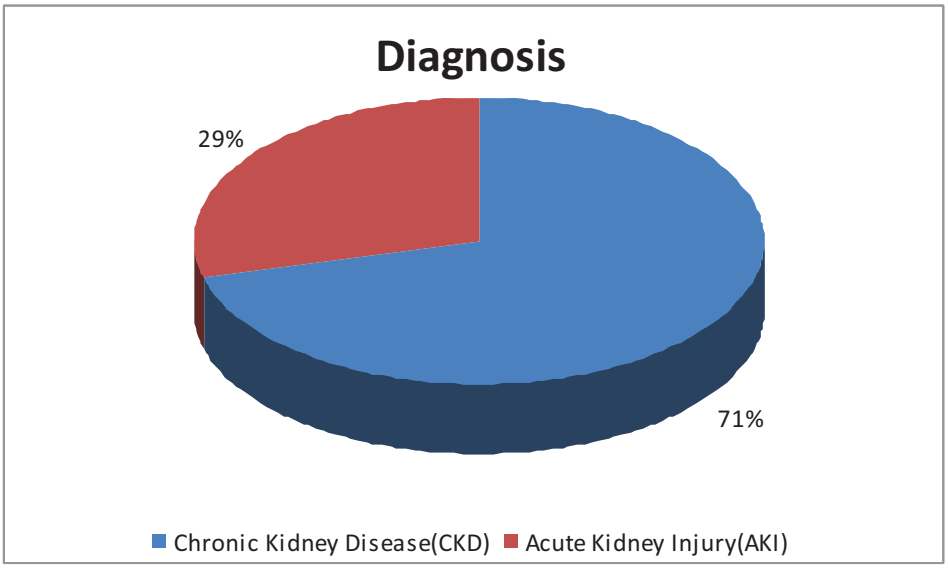


Fig 2: Diagnosis

TABLE 3: INDICATION FOR HEMODIALYSIS IN THE STUDY GROUP (n= 150)

Acute Kidney Injury	**No**	**%**	**Chronic Kidney Disease**	**No**	**%**
Medical renal disease < 3months	22	50.0	Diabetes Mellitus	37	35.0
Acute diarrheal disease	7	16.1	Hypertension	34	32.0
Snake Bite	3	6.8	Post renal transplant failure	15	14.2
Postpartum	3	6.8	End Stage Renal Diseases(ESRD)	8	7.5
Post surgical	3	6.8	Anaemia with Failure	4	3.9
Drug induced	2	4.6	Nephrotic Syndrome	3	2.8
Copper Sulphate poisoning	2	4.6	Systemic lupus erythematosis	3	2.8
Hemolytic Jaundice	1	2.2	Hemolytic uremic syndrome	1	0.9
Hydronephrosis	1	2.2	Renal artery stenosis	1	0.9
Total	**44**	**29.3%**	**Total**	**106**	**70.7%**

The predominant cause among patient with CKD was Diabetes mellitus(35%), followed by Hypertension(32%). 14.2% patients had post renal transplant failure. Among the 44 of AKI 50% had Medical renal disease less than 3 months and 16.1% had acute diarrheal disease.

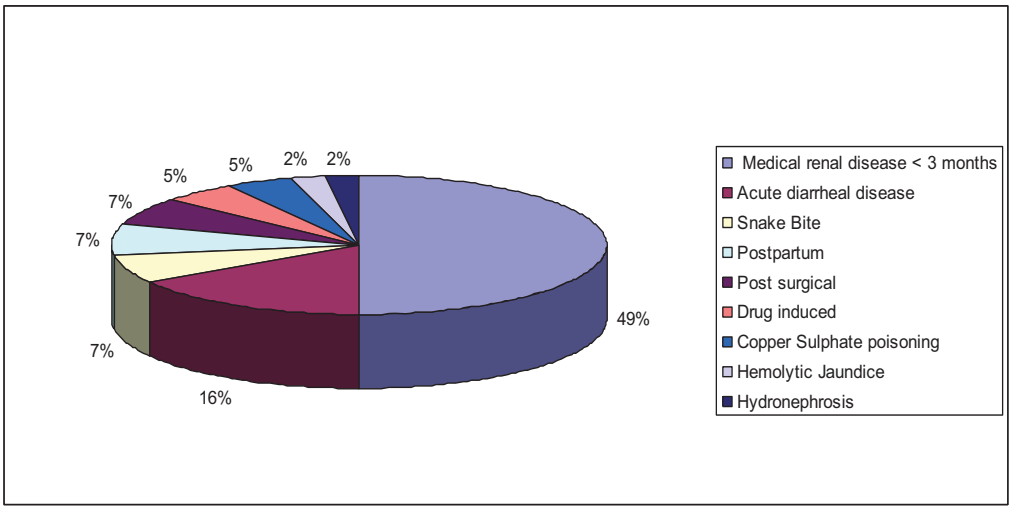


Fig 3: Acute Kidney Injury

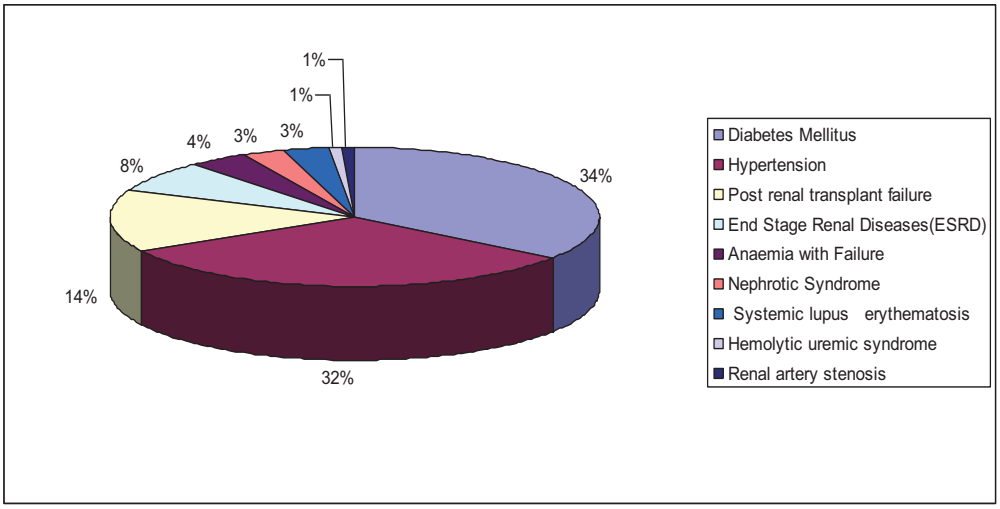


Fig 3(a): Chronic Kidney Disease

TABLE 4: DISTRIBUTION OF SAMPLES COLLECTED FROM STUDY GROUP AND THEIR CULTURE POSITIVITY

Samples		Aerobic Bacteria	Fungi	Parasites
Catheter tip	(n= 150)	88 (58.3%)	7 (4.6%)	-
Peripheral venous blood	(n= 150)	35 (23.3%)	3 (2%)	
Central venous catheter blood	(n=9)	6 (66.6%)	-	-
Swab from Infected Site	(n=12)	4 (33.3%)	-	-
Urine	(n=33)	11 (33.3%)	8 (24.2%)	
Sputum	(n=15)	6 (40%)	-	-
Stool	(n=20)	-	-	13 (65%)

Catheter tip and peripheral venous blood was collected from all the cases. Central venous catheter blood sample was collected in 9 cases along with the peripheral blood. None of the cases with urinary infection or upper respiratory infection had a positive culture in peripheral venous blood.

58.3% and 23.3% of Aerobic bacteria were isolated from catheter tip and peripheral venous blood samples respectively. Fungi isolated from 2% of peripheral venous blood samples and 24% of urine samples. Parasitic infections was noted in 65% of patient with gastrointestinal symptoms.

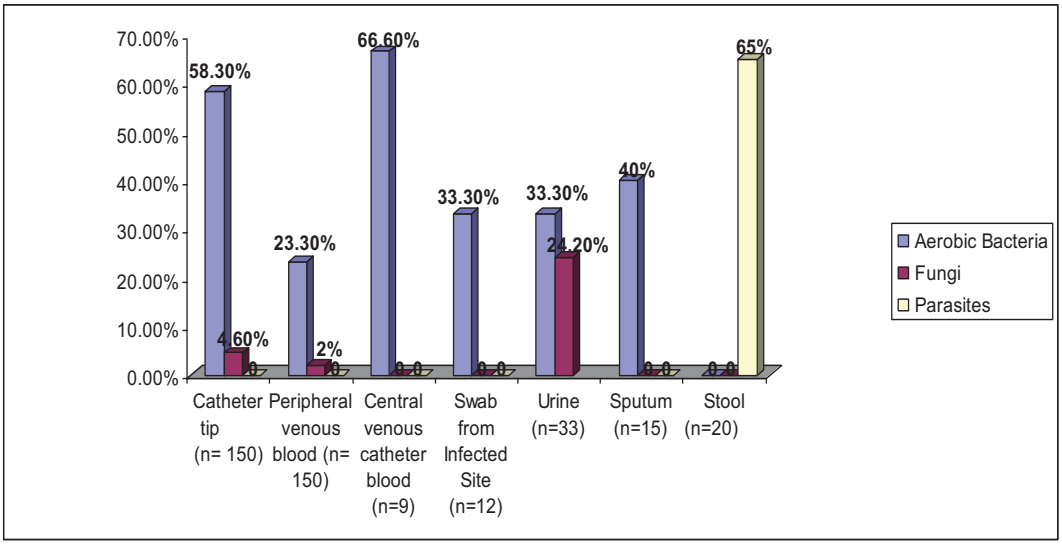


Fig 4: Distribution of samples collected from study group and their culture positivity

TABLE 5: DIRECT GRAM STAINING OF CATHETER TIP

Findings	Number	Percentage
Gram Positive Organism with pus cells	27	18.0 %
Gram Negative Organism with pus cells	11	7.4%
Presence of Polymorphs	35	23.3 %
No specific findings	77	51.3 %

Gram staining demonstrated Gram positive cocci in 18% and Gram negative bacilli in 7.4% of cases. Polymorphs were seen in 23.3% catheter samples

TABLE 6: CULTURE POSITIVITY OF CATHETER TIP BY VARIOUS CULTURE METHODS (n=150)

Micro organism	Roll Plate Method	Vortex	Segment Washing
Culture Positive	88(58.7%)	71(47.4%)	28 (18.7%)
No Growth	62(41.3%)	79(52.6%)	122(81.3%)

Maximum culture positivity was observed in roll plate method 88 (58.7%), Compared to other methods like Vortex and segment washing which showed 71(47.4%) and 28(18.7%) of culture positivity respectively.

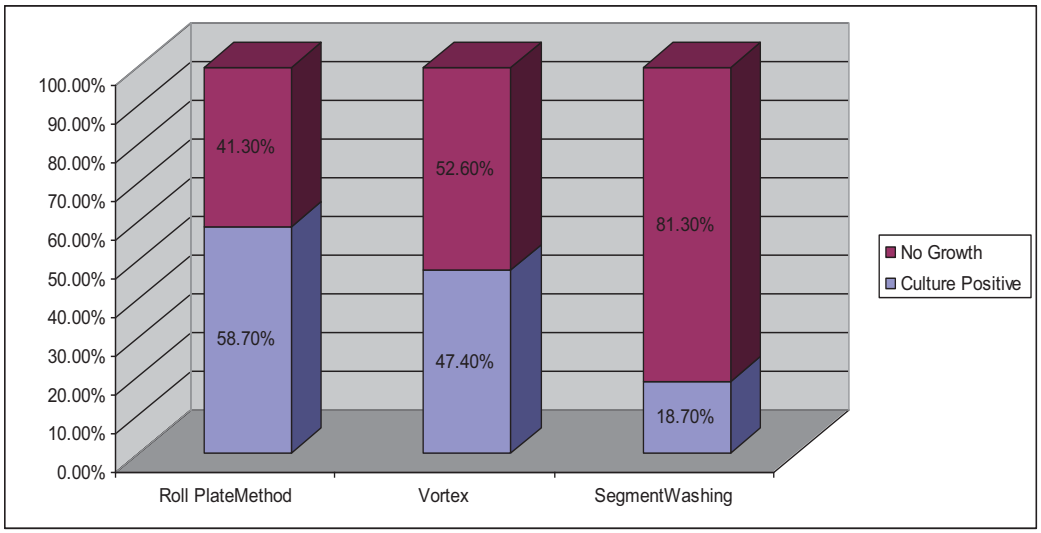


fig 5: Culture positivity of catheter tip by various culture methods (n=150)

TABLE 7: QUANTITATIVE BACTERIAL CULTURE OF CATHETER TIP & PERIPHERAL VENOUS BLOOD

Colony Forming Unit/ ml	Catheter tip	Peripheral Blood
Nil Count	62 (41.3%)	115 (76.7%)
$> (10)^2$ col/ml	88 (58.7%)	35 (23.3%)

Among the positive culture in catheter tip and peripheral blood colony count of $> 10^2$cfu/ml was observed in 88 cases (58.7%) and 35 cases (23.3%) respectively.

Chisquare value = 38.707, $p<0.05$ - statistically significant

TABLE 8: CATEGORIZATION OF CATHETER RELATED INFECTION

Catheter related Infection	Number	Percentage
Catheter related infection without positive blood culture	53	35.3 %
Catheter related infection with positive blood culture	35	23.3%
Negative culture for both catheter and peripheral venous blood	62	41.4 %

Among the 88 catheters processed 53(35.3%) was catheter culture positive but blood culture negative, hence categorized as catheter related infection(CRI). But 35 cases (23.3%) was positive for both catheter and blood culture and showed similar growth in both, therefore categorized as Catheter related blood stream infection(CRBSI).

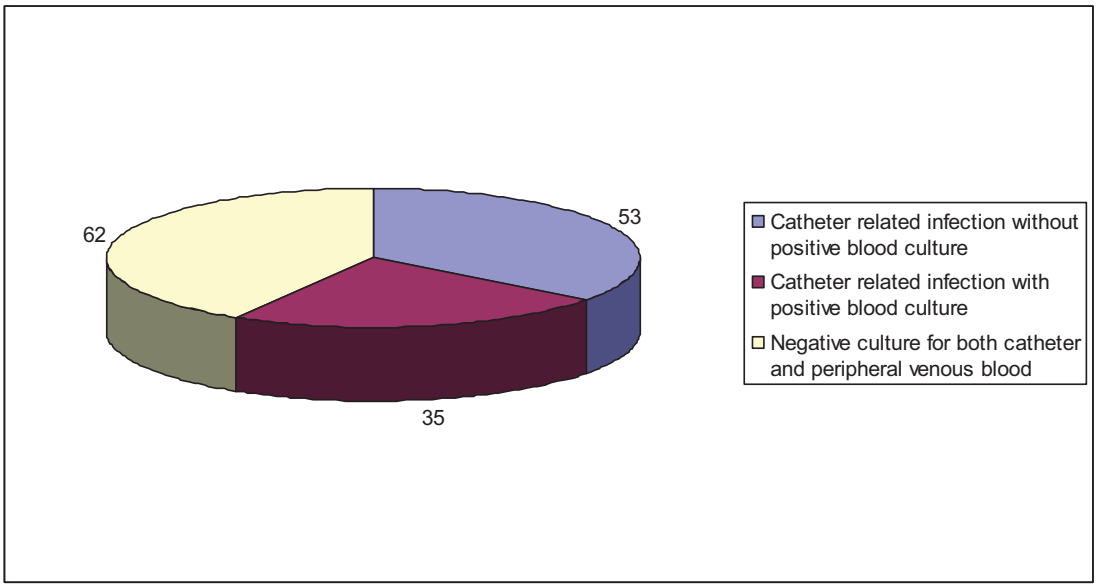


fig 6: categorization of Catheter related infection

TABLE 9: CORRELATION OF CRBSI WITH AGE

Age in years	No.of Cases	Positive cultures	Percentage of positive culture
18-20	6	1	16.6
21-30	51	14	27.4
31-40	30	6	20.0
41-50	40	7	17.5
51-60	15	5	33.3
>60	8	2	25.0

CRBSI was noted most commonly in the age group of 51-60 years (33.3%) followed by 27.4% between 21-30 yrs.

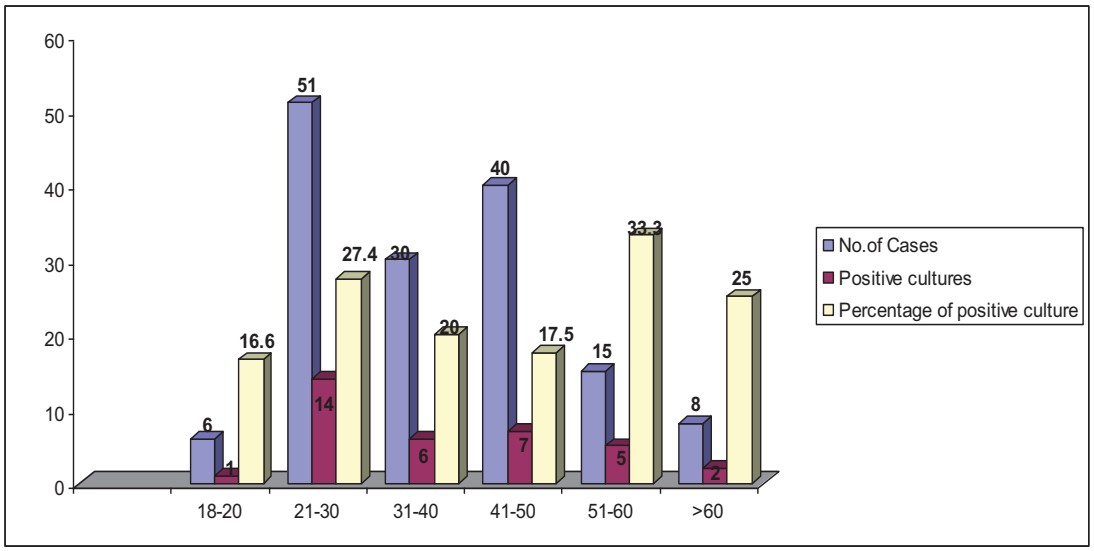


fig 7: Correlation of catheter related blood stream infection with age

TABLE 10: ASSOCIATION BETWEEN SITE OF CATHETERIZATION AND CRBSI

Site	No.of Cases	Positive cultures	Percentage
Internal Jugular vein	144	30	20.8
Subclavian vein	3	2	66.6
Femoral vein	3	3	100

The commonest site of central venous catheterization was the internal jugular vein. CRBSI was detected in 20.8% of these catheters. Femoral venous catheterization was done in very few patients and they have the higher risk of developing CRBSI.

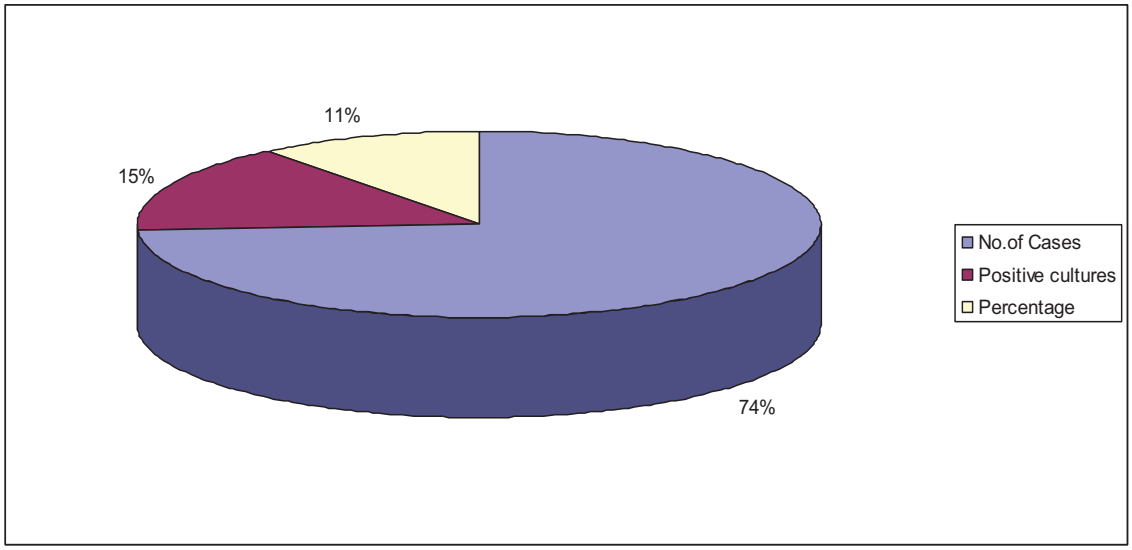


fig 8: Association between site of Catheterization and CRBSI

TABLE 11: CORRELATION BETWEEN NUMBER OF DIALYSIS AND POSITIVE AEROBIC BACTERIAL CULTURES IN CRBSI

Number of dialysis	No.of Cases	Positive cultures	Percentage of positive culture
1-5	96	9	9.3
6-10	29	12	41.3
>10	25	14	56.0

As the number of dialysis increased, the culture positivity also increased. Patients underwent HD more than 10 times showed 56 % of Catheter related blood stream infection.

Chisquare =30.648, $p<0.05$ - statistically significant.

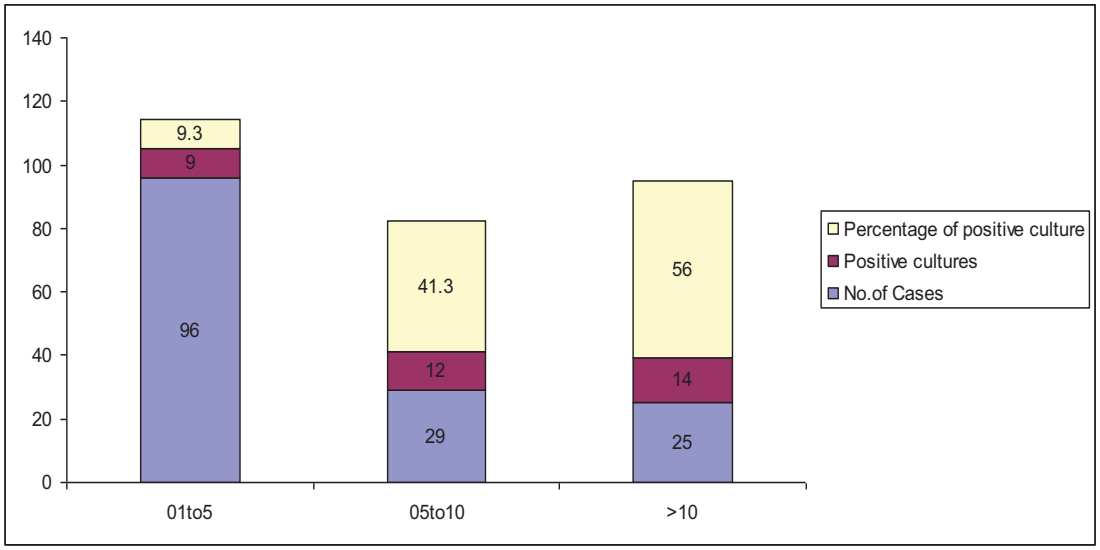


fig 9: Correlation between number of dialysis and positive aerobic bacterial cultures in CRBSI

TABLE 12: ASSOCIATION BETWEEN DURATION OF CATHETERIZATION AND CRBSI

Duration of Catheterization	NO	Percentage
1-5 days	1	2.8
6-10 days	4	11.4
11-15 days	12	34.3
>15 days	18	51.4

The frequency of CRBSI was 51.4% in patients with catheter in place for >15 days followed by 34.3% when the duration was between11-15 days.

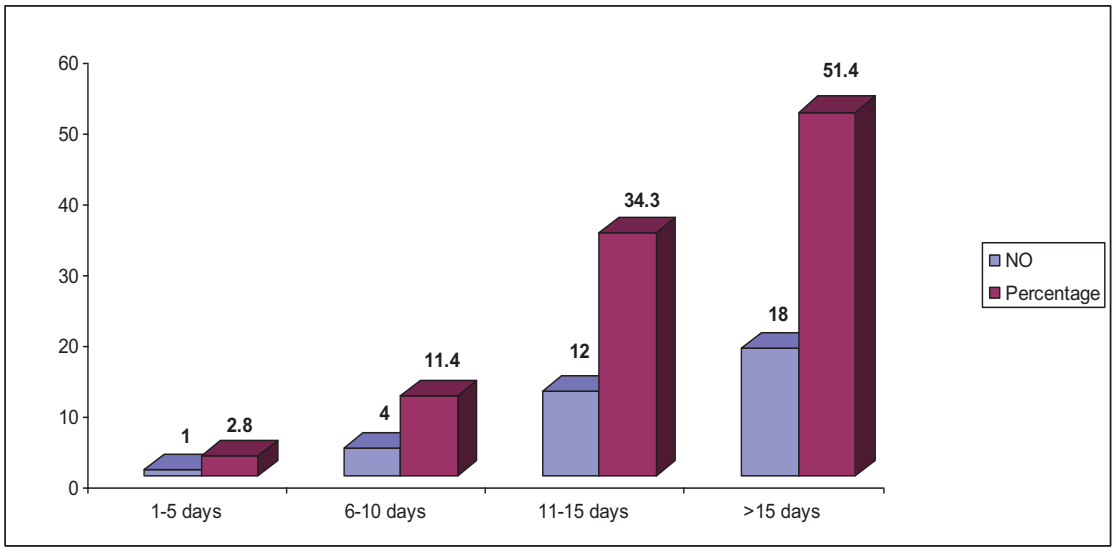


fig 10: Association between duration of catheterization and CRBSI

TABLE 13: TYPE OF PREDISPOSING FACTORS FOR CRBSI

Diagnosis	No of cases	Percentage
History of previous dialysis	31	88.5
History of infection in previous dialysis	28	80.0
Anaemia	32	91.4
Diabetes Mellitus	33	94.2
Hypertension	11	31.4
Hepatitis C positive	1	2.8

Among the 35 cases of CRBSI predominant risk factor observed was diabetes mellitus 94.2%,followed by anaemia 91.4% .

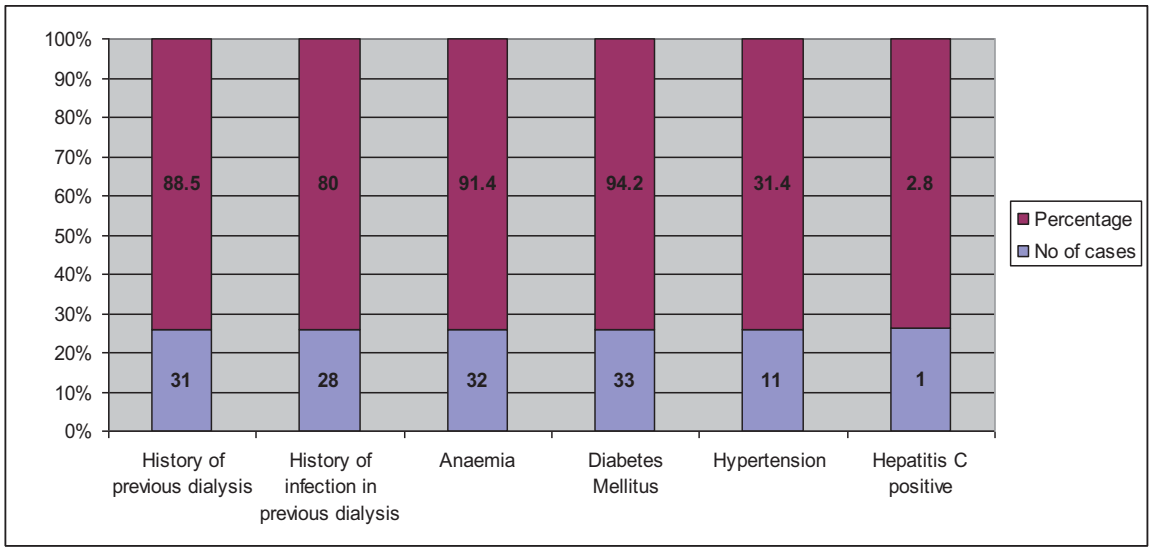


fig 11: Type of predisposing factors of CRBSI

TABLE 14: DISTRIBUTION OF AEROBIC BACTERIAL ISOLATES FROM VARIOUS SAMPLES

ISOLATE	Catheter (n=88)	Peripheral blood (n=35)	Catheter blood (n=6)	Urine (n=11)	Stool	Swab 4	Sputum 6
S.aureus MRSA	24 (27.2%)	11 (31.4%)	2(33.3%)		-		
MSSA	5 (5.6%)	3 (8.5%)	-	-	-	1	3 (50%)
S.epidermidis	23 (26.1%)	3 (8.5%)	-	3 (27.2%)	-		
S.warneri	2 (2.2%)	-	-	-	-		
S.schleiferi	2 (2.2%)	2 (5.7%)	-	-	-		
Micrococcus Spp	4 (4.5%)	-	-	-	-		
Pseudomonas aeruginosa	7 (8.0%)	5 (14.2%)	1 (16.7%)	-	-		
Acinetobacter baumannii	8(9.0%)	4 (11.4%)	3 (50.0%)	-	-		
E.coli	4 (4.5%)	3 (8.5%)		8 (72.2%)	-	1 (25%)	
K.oxytoca	3 (3.4%)	2 (5.7%)			-	2 (50%)	
K.pneumoniae	2 (2.2%)	1 (2.8%)			-		3 (50%)
Proteus mirabilis	2 (2.2%)	1 (2.8%)			-		
Proteus vulgaris	2 (2.2%)	-			-		

- *Staphylococcus aureus* was the predominantly isolated gram positive cocci (GPC) from catheter and peripheral venous blood. *Pseudomonas aeruginosa* was the commonest gram negative bacilli isolated from catheter tip.

- Methicillin resistance was noted among *Staphyloccus aureus* isolates in 27.2% and 31.4% catheter tip and peripheral venous blood isolates.

- 33.3% MRSA, 16.7% *Pseudomonas aeruginosa* and 50% *Acinetobacter baumannii* isolated from catheter blood.

- The commonest etiological agent in urinary tract infection was Escherichia coli (72.2%)

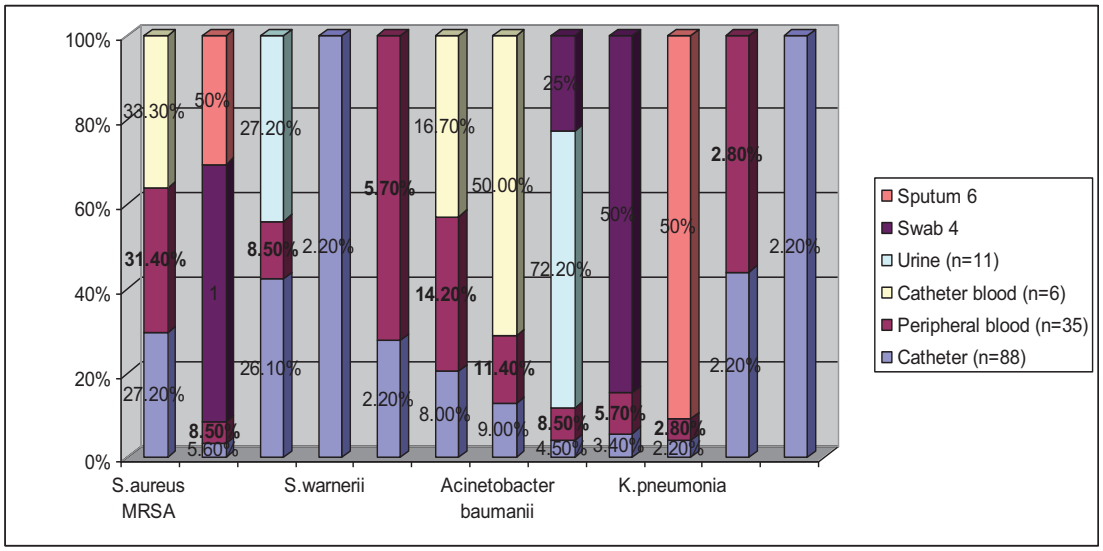


fig 12: Distribution of Aerobic Bacterial isolates from various samples

TABLE 15: ETIOLOGICAL AGENTS OF CRBSI (n=35)

Isolate	CRBSI(35)
S.aureus MRSA	11 (31.4%)
MSSA	3 (8.5%)
S.epidermidis	3 (8.5%)
S.schleiferi	2 (5.7%)
Pseudomonas aeruginosa	5(14.2%)
Acinetobacter baumanii	4 (11.4%)
E.coli	3 (8.5%)
K.oxytoca	2 (5.7%)
K.pneumoniae	1 (2.8%)
Proteus mirabilis	1 (2.8%)

Among the gram positive organism, predominant isolate was 31.4% of Methicillin resistant *Staphylococcus aureus* followed by Methicillin sensitive *Staphylococcus aureus* (8.5%).Among the gram negative organism *Pseudomonas aeruginosa* was the etiological agent (14.2%), followed by *Acinetobacter baumannii* (11.4%).

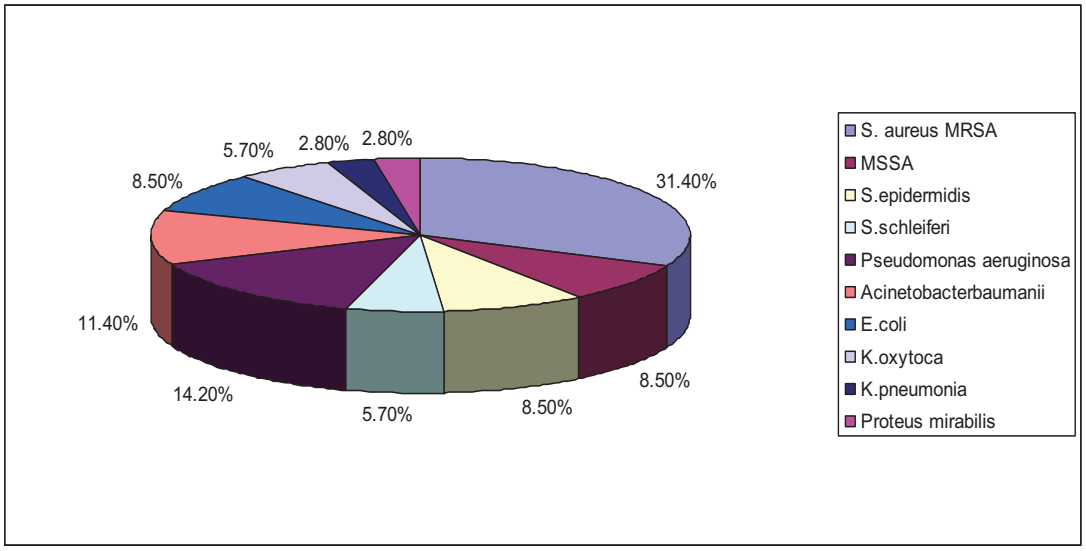


fig 13: Etiological Agents of CRBSI

TABLE 16: TYPE OF POLY MICROBIAL INFECTION IN CRBSI (n=35)

Mixed Growth	Catheter
Staphylococcus aureus + Candida albicans	4 (11.4%)
Staphylococcus aureus + Klebsiella oxytoca	2 (5.7%)

Among the total of 6 cases of polymicrobial growth, *Staphylococcus aureus* and *Candida* species was isolated in 11.4% followed by *Staphylococcus aureus* and *Klebsiella oxytoca* in 5.7%.

TABLE 17: CORRELATION OF CRBSI AND DEVELOPMENT OF COMPLICATIONS

Complication	No.of Cases	Percentage
Hypotension	4	11.4%
Septic Thrombophlebitis	2	5.7 %
Death	1	2.8 %

Among the 35 cases of CRBSI, 11.4% developed hypotension and 5.7% septic thrombophlebitis. Mortality was seen in 2.8% cases.

TABLE 18: TYPE OF FUNGAL ISOLATES FROM VARIOUS SAMPLES (n=18)

Fungi	Catheter tip	Peripheral venous lood	Urine
C.albicans	2 (11.1)	1 (5.6)	4 (22.2)
C.parapsilosis	1 (5.6)	1 (5.6)	1 (5.6)
C.tropicalis	1 (5.6)	1 (5.6)	3 (16.6)
Asp. Flavus	2 (11.1)	-	-
Asp.fumigatus	1 (5.6)	-	-

A total of 5.6% of *C.albicans, C.tropicalis, C.parapsilosis* was isolated from blood. *Aspergillus flavus* was isolated only from catheter in 11.1% of cases and *Aspergillus fumigatus* in 5.6% of cases. Among the 4 cases *Candida* species isolated from catheter 3 cases had candidaemia.

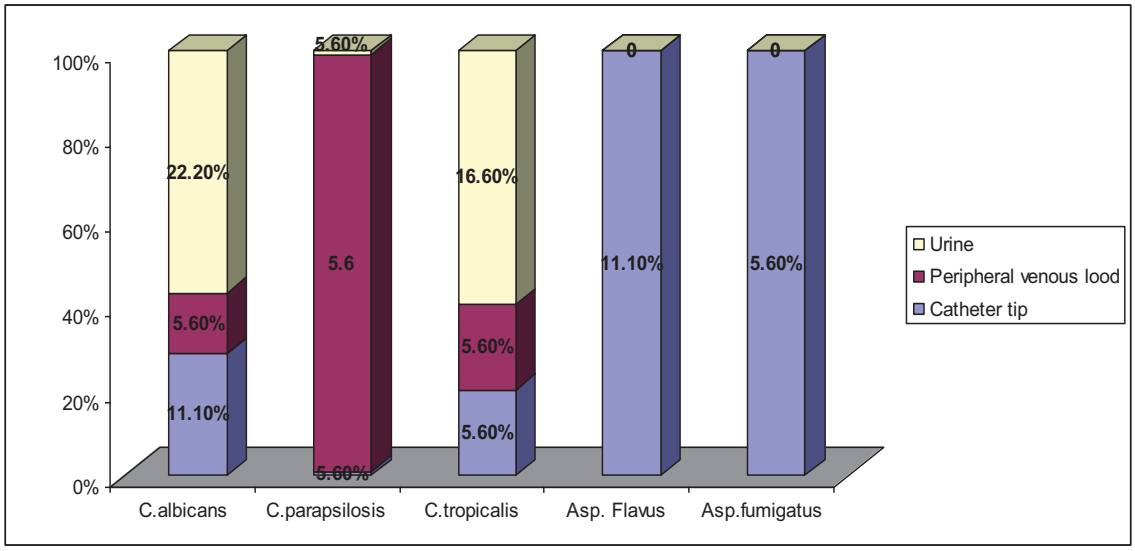


fig 14: Type of fungal isolates from various samples

TABLE 19: TYPE OF PARASITES FROM STOOL SAMPLE (n=13)

Parasite	No	Percentage
Entamoeba histolytica	6	46.1%
Giardia lamblia	4	30.7%
Cryptosporidium parvum	2	15.3%
Microsporidia	1	7.6%

Entamoeba histolytica was predominantly seen in 46.1% of patients with gasterointestinal symptoms followed by *Giardia* 30.7% and *Cryptosporidium parvum* 15.3%.

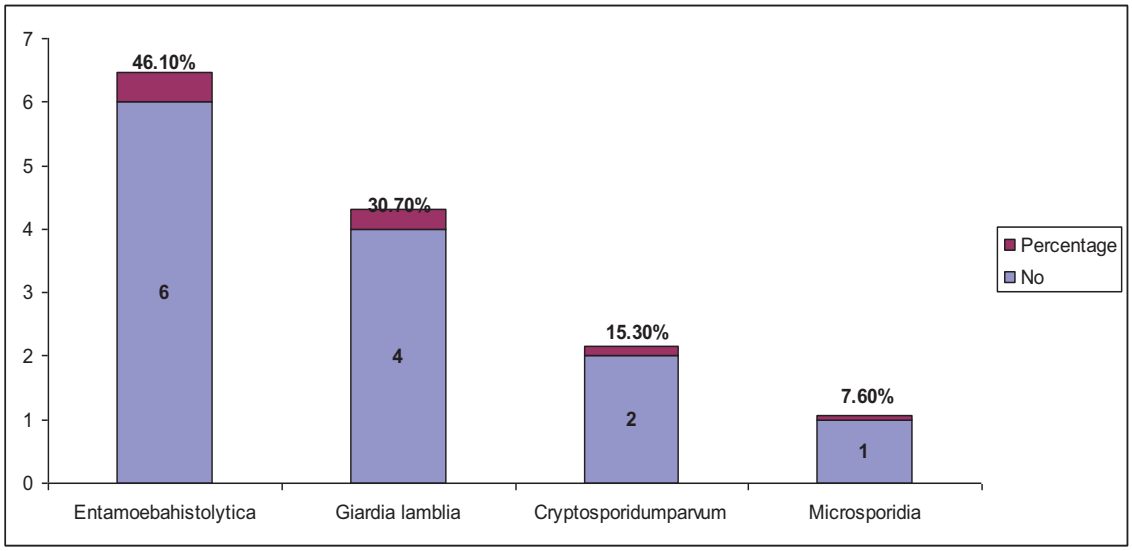


Fig 15: Type of Parasites from Stool Sample

TABLE 20: ANTIMICROBIAL SENSITIVITY PATTERNS OF GRAM POSITIVE COCCI (GPC)

Antibiotics	*Staphylococcus aureus* (n=48)										*Staphylococcus epidermidis*(n=29)						*Staphylococcus schleiferi*(n=4)				*Staphylococcus warneri* (n=2)	
	Catheter tip(n=29)		Peripheral venous blood(n=14)		Catheter Blood(n=2)		Sputum(n=3)		Swab(n=1)		Catheter tip(n=23)		Peripheral venous blood(n=3)		Urine (n=3)		Catheter tip(n=2)		Peripheral venous blood(n=2)		Catheter tip(n=2)	
	N	%	n	%	N	%	n	%	n	%	N	%	n	%	n	%	n	%	N	%	n	%
Amikacin	29	100	14	100	2	100	3	100	1	100	23	100	3	100	3	100	2	100	2	100	2	100
Ciprofloxacin	22	75	10	71.4	1	50	3	100	1	100	18	78	2	67	3	100	2	100	1	50	1	50
Cotrimoxazole	21	72	11	100	2	100	2	67	-	-	19	83	2	67	3	100	1	50	1	50	1	50
Cephalexin	22	76	8	57	2	100	1	33	-	-	19	83	1	33	3	100	1	50	1	50	2	100
Ofloxacin	22	76	8	57	2	100	1	33	1	100	19	83	2	67	3	100	1	50	1	50	2	100
Erythromycin	22	76	10	71	1	50	3	100	1	100	18	78	2	67	3	100	2	100	1	50	1	50
Penicillin	-	-	-	-	-	-	3	100	1	100	18	78	2	67	3	100	2	100	1	50	1	50
Amoxycycillin clavulunic acid	24	100	11	100	2	100	3	100	1	100	23	100	3	100	3	100	2	100	2	100	2	100
Cefoxitin	5	17.2	3	21.4	-	-	3	100	1	100	23	100	3	100	3	100	2	100	2	100	2	100

Staphylococcus aureus, *Staphylococcus epidermidis*, *Staphylococcus schleiferi* and *Staphylococcus warneri* showed 100% sensitive to Amikacin. 27.2%, 31.4%, 33.3% of MRSA were detected in catheter tip, peripheral venous blood and catheter blood respectively.

TABLE 21: ANTIMICROBIAL SENSITIVITY PATTERNS OF GRAM NEGATIVE BACILLI (GNB)

Antibiotic	*Pseudomonas aeruginosa* n=13						*Acinetobacter baumannii* n=15					
	Catheter tip(n=7)		Peripheral Blood(n=5)		Catheter Blood(n=1)		Catheter tip(n=8)		Peripheral Blood(n=4)		Catheter Blood(n=3)	
	N	%	N	%	N	%	N	%	N	%	N	%
Amikacin	7	100	4	80	1	100	5	62.5	4	100	3	100
Gentamicin	6	85.6	4	80	1	100	6	62.3	4	100	3	100
Ceftazidime	7	100	5	100	1	100	8	100	4	100	3	100
Ciprofloxacin	6	85.6	4	80	1	100	7	87.5	4	100	2	66.6
Ofloxacin	7	100	5	100	1	100	8	100	4	100	3	100
Imipenem	7	100	5	100	1	100	8	100	4	100	3	100
Cefoperazone /sulbactam	7	100	5	100	1	100	8	100	4	100	3	100
Pipracillin /Tazobactum	7	100	5	100	1	100	8	100	4	100	3	100

All the isolates of *Pseudomonas aeruginosa and Acinetobacter baumannii* showed 100% sensitive to Imipenam.No MBL, AmpC producers were detected.

TABLE 22: ANTIMICROBIAL SENSITIVITY PATTERNS OF GRAM NEGATIVE BACILLI (GNB)

Antibiotic	*Klebsiella pneumoniae* (n=6)						*Klebsiella oxytoca* (n=7)						*Proteus mirabilis* (n=3)				*Proteus vulgaris* (n=2)		*Escherichia coli* (n=16)							
	Catheter tip(n=2)		Peripheral Blood (n=1)		Sputum (n=3)		Catheter tip(n=3)		Peripheral Blood(n=2)		Swab(n=2)		Catheter tip(n=2)		Peripheral Blood (n=1)		Catheter tip(n=2)		Catheter tip(n=4)		Peripheral Blood(n=3)		Swab(n=1)		Urine (n=8)	
	n	%	n	%	n	%	n	%	N	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Amikacin	2	100	1	100	3	100	3	100	2	100	2	100	2	100	1	100	2	100	4	100	3	100	1	100	8	100
Gentamicin	2	100	1	100	3	100	2	66.6	2	100	2	100	2	100	1	100	2	100	4	100	3	100	1	100	8	100
Cefotaxime	1	50	-	-	2	66.6	2	66.6	1	50	2	100	1	50	1	100	1	50	2	50	1	33.3	-		4	50
Ceftazidime	2	100	1	100	3	100	3	66.6	2	100	2	100	1	50	1	100	1	50	2	50	1	33.3	-		4	50
Ciprofloxacin	2	100	1	100	1	100	3	100	2	100	1	100	2	100	1	100	2	100	3	75	1	33.3	-	100	8	100
Ofloxacin	2	100	1	100	3	100	3	100	2	100	2	100	2	100	1	100	2	100	4	100	3	100	1	100	8	100
Imipenem	2	100	1	100	3	100	3	100	2	100	2	100	2	100	1	100	2	100	4	100	3	100	1	100	8	100
Cefoperazone /sulbactam	2	100	1	100	3	100	3	100	2	100	2	100	2	100	1	100	2	100	4	100	3	100		100	8	100
Nitrofurantoin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	100
Norfeox	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	100

All the members of Enterobacteriaceae were sensitive to Imipinem and Cefoperazone/sulbactam.33.3% of *Proteus mirabilis,* 37.5% of *E-coli*, 16.6% *Klebsiella pneumoniae*, 50% of *Proteus vulgaris* and 14.2% *Klebsiella oxytoca* were ESBL producers

TABLE 23: MIC OF VANCOMYCIN FOR *STAPHYLOCOCCUS AUREUS*

Organism	Minimum inhibitory concentration Break Point			
	0.25 µg/ml	0.5 µg/ml	1.00 µg/ml	>2 µg/ml
S.aureus n=37	22	15	-	-

Among the 38 isolates of MRSA all were sensitive to vancomycin with the MIC of <2.

TABLE 24: DETECTION OF ESBL PRODUCERS AMONG ZTHE GRAM NEGATIVE BACTERIA FROM VARIOUS SAMPLES

	Number of positive isolates					
Pathogens	Screening test		DDST		PCDDT	
Proteus mirabilis(n=3)	2	66.6%	1	33.3%	1	33.3%
Escherichia coli (n=16)	7	43.75%	6	37.5%	6	37.5%
Klebsiella pneumoniae (n=6)	3	50.0%	1	16.6%	1	16.6%
Proteus vulgaris (n=2)	1	50.0%	1	50%	1	50%
Klebsiella oxytoca (n=7)	2	28.5%	1	14.2%	1	14.2%

PCDDT-Phenotypic confirmation disk diffusion test, DDST-Double disk diffusion synergy test.16.6% of *Klebsiella pneumoniae*,50% of *Proteus vulgaris*, 33.3% of *Proteus mirabilis* and 37.5% of *Escherichia coli* and 14.2% of *Klebsiella oxytoca* were found to be ESBL producers.

TABLE 25: ANTI FUNGAL SUSCEPTIBILITY PATTERN OF CANDIDA ISOLATES

Isolate	No. of Isolates	Fluconazole		Amphotericin B	
		Sensitive	Resistance	Sensitive	Resistance
		MIC ≤ 8	MIC>64	MIC ≤1	MIC>1
Candida albicans	7	7 (100)	-	7(100)	-
Candida parapsilosis	3	3 (100)	-	3(100)	-
Candida tropicalis	5	5 (100)	-	3(100)	-

All the *Candida* isolates were 100% sensitive to Fluconazole and Amphotericin B .

TABLE 26: ANTI FUNGAL SUSCEPTIBILITY PATTERN OF ASPERGILLUS SPECIES

Isolate	Amphotericin B		Itraconazole		Voriconazole	
	Sensitive MIC≤2	Resistance MIC>2	Sensitive MIC≤8	Resistance MIC>8	Sensitive MIC≤8	Resistance MIC>8
Aspergillus flavus (n=2)	2 (100)	-	2 (100)	-	2 (100)	-
Aspergillus fumigatus (n=1)	1 (100)	-	1 (100)	-	1 (100)	-

Asp.flavus and *Asp.fumigatus* showed 100% sensitive to Amphotericin B, Itraconazole, Voriconazole

DISCUSSION

This study was conducted in the Institute of Microbiology is association with Department of Nephrology, Rajiv Gandhi Government General Hospital, Chennai.

The analysis of catheter related sepsis in hemodialysis patients needs a careful microbiological evaluation to differentiate between, Colonization Catheter related infection (CRI) and Catheter related blood stream infection (CRBSI). The prime interest of this study was to evaluate the central venous catheter related bacterial and fungal infection associated with CRBSI and also to screen the other bacterial, fungal and parasitic infection in patients on hemodialysis. Careful monitoring and management of catheter related blood stream infection will in turn reduce the rate of occurrence of nosocomial infection in our tertiary care hospitals.

None of the patients included in our study falls under the category of Colonization, as the specimens were collected only from symptomatic patients after hospitalization.

In our study 150 adult inpatients who underwent hemodialysis through the central venous catheter with symptoms and signs of infection were taken. Majority of the study population were males (52%) and females (48%) with the ratio was 1.8:1. Majority (67.4%) were between the age group of 21-30yrs[Table1]. Hemodialysis was performed through central venous catheters in 70.7% of Chronic kidney diseases (CKD) and 29.3% of Acute kidney injury(AKI) patients.[Table 2] Among the Chronic kidney disease patients(CKD) Diabetes Mellitus was the predominant underlying cause of catheterization in 35%

followed by hypertension 32%, post renal failure 14.2% and end stage renal disease in 7.5%. Less than 5% contributed by anaemia with failure, nephrotic syndrome, hydronephrosis, hemolytic uremic syndrome and systemic lupus erythematosis. In Acute kidney injury (AKI) medical renal disease less than 3 months contributed 50% followed by 16% diarrhea, 6.8% snake bite, post partum, and post surgical causes.4.6% had history of poisoning and 2.2% had hemolytic anaemia.[Table 3]

Peripheral venous blood and catheter tip were collected from all 150 patients and rest of the samples like swab from the infected site, urine, sputum and stool were collected when clinically indicated.[Table4].

Out of the 150 catheters subjected to direct grams stain demonstrated 18.0% gram positive organisms with pus cells, (7.4%) showed gram negative organisms with pus cells and (23.3%) of the catheters showed presence of polymorphs with no organisms. Catheters which showed organisms in direct gram stain also showed culture positivity in 80% cases. The remaining 20% of catheter was gram stain positive and culture negative but those patients had history of antibiotics prior to catheter removal. Peter *et al*.[98] reported 20% catheter tip gram staining had pus cells with organisms which was similar to our study.[Table 5].

Demonstration of catheter tip culture is usually performed by Maki's semiquantitative method which is a reference standard for more than 25 years.[34] In our study we found that Maki's technique was superior than other quantitative methods. 58.6% showed culture positivity by roll plate method. 47.4% of the catheter culture positivity was detected by vortex method and

18.7% by segment washing method.[Table6] Our study was similar to the study of Emilio *et al*.[27] who stated 98% of the catheter related infections were detected by roll plate method, therefore reported that roll plate method was best for detecting catheter related infections.[78,79,80]

The colony count of $>10^2$cfu/ml was detected by quantitative culture of catheter tip and peripheral venous blood in 58.7% and 23.3% respectively (p Value <0.005) which is statistically significant.[Table7] Our study matches with study by Emilio *et al*.[27] that majority of catheter related infection were detected only by the roll plate method than quantitative culture. Our study correlates with the study of Siegman *et al*.[81]. Who showed that Maki's technique was superior than other methods in detecting catheter related infections.

Our study the rate of occurrence CRBSI was noted 23.3% of cases and CRI in 35.5% of cases.[Table 8] Catheter related infection was the underlying cause for the development of Catheter related blood stream infection in the course of hemodialysis and further complications like thromophlebitis, endocarditis and septic arthritis. Biofilm forming organisms like *S.epidermidis* and *E.coli* in catheters inturn may worsen the condition of the patient. The rate of occurance of CRBSI in our study was slightly higher than the study of Pooja *et al*.[2] who reported 15% of CRBSI. However our study correlates with the study of Ratnaja *et al*., and Anil *et al*.,[Table 8] that they reported CRBSI in 12% of cases. Ramanathan *et al*. [56] reported CRBSI in 40% of cases..

In our study patients in the age group between 51-60 years showed more frequency of CRBSI (33.3%) followed by in those with the age group

of 18-20 years (27.4%.).[Table 9] Our study correlates with the study of Sexana *et al*.[23] that elderly patients are more prone to develop CRBSI. Pooja *et al.* reported 16% of CRBSI occurred between the age group of 15-30yrs, 20.5% in 31-45yrs and 9.1% in 46-60yrs[2] . Keiren *et al*. reported 40% incidence of CRBSI above 55 year of age.[91]

Among the 150 catheters internal jugular vein was the commonly catheterized site. 20.8% of internal jugular venous catheters had CRBSI subclavian and femoral were inserted only in 3 patients each and showed 66.6% and 100% culture positivity respectively.[Table10] Pooja *et al*[2], described 12% of culture positivity through internal jugular vein, 22.2% through subclavian vein. Merrer *et al*[71]. noted higher incidence of infection at the femoral venous site, compare to subclavian vein. Goetz *et al.*[72] also reported that the risk of infection with femoral venous site was more particularly if it was inserted as an emergency and as well as in post renal transplantation(72%). Mermal *et al*[73], described that due to the close proximity to oropharygeal secretions, difficulty in monitoring the sterile dressing and internal jugular site, the risk of infection was more at IJV. According to Harsha *et al.*[26] the incidence of catheter infection was 33.3% in subclavian vein, 23.8% in femoral and 25% in IJV. Ratnaja *et al*.[4] reported that rate of catheter infection in femoral, IJV and subclavian was 7.6%, 5.6% and 0.7% respectively. Anil.K.Sexsana *et al*.[23]stated that infection through femoral vein was 7.6 episodes and internal jugular vein was 2.7 episodes per 1000 catheter days. Our study correlates with the study of Harsha *et al*.[26] that more incidence of CRBSI was due to catheterization through subclavian vein.

European Renal Best Practice ERBP[52] recommends right IJV is the preferred site of catheterization followed by left IJV, the rate of stenosis in subclavian was more and the rate of infection was more in the femoral catheterization. Hence the use of femoral and subclavian catheterization was discouraged by ERBP.

In our study patients who underwent HD less than 5 times showed 9.3% of developing CRBSI and those patients hemodialysis between 5-10 times showed 41.3% and more than 10 times showed 56% of developing CRBSI. Patients in whom the catheter was placed in site more than 15 days had 51.4% CRBSI (p Value<0.005) which is statistically significant.

Our study also found that CRBSI was increased as the number of dialysis increased. This was similar to the study of Ramanathan *et al.*[56], Harsha *et al.*[26] who reported that CRBSI is significant among patients with catheter more than 3 days. Eyes *et al.*[74] found that patients with longer duration more than 7 days of catheterization were more prone to develop bacteremia.[Table 11-12]

Among the risk factors analyzed, CRBSI was seen in 94.2% of patients with diabetes mellitus, 91.45% in anaemia, 88.5% in patients with history of previous dialysis and 2.8% cases with Hepatitis C infection. Our study correlates with the study of Powe *et al.*[95] who reported that anaemia, and diabetes mellitus was the predominant risk factors associated in patients with CRBSI. Hoen *et al.*[77] Robert *et al.*[85] also stated that 12-16% of patients with CRBSI had anaemia and diabetes mellitus. Allon *et al*[86] Jonathan *et at*[96] and Rose *et al* [14] had reported that anaemia was the major underlying cause of patients with

CRBSI . Surendrakumar *et al* [82]Chandra *et al* [83] Reddy *et al* [84] reported that Hepatitis C infection was prevalent in patients undergoing maintenance HD and the risk of developing CRBSI in 6 -7% of cases.[Table 13].

Among the gram positive organisms isolated from catheter tip Methicillin resistant *Staphylococcus aureus* (MRSA) was the predominant organism in (27.7%) followed by *Staphylococcus epidermidis* (26.1%). Our study correlates with the study of Pooja *et al*[2] who stated that 67% of the CRBSI was due to gram positive cocci(33%)contributed by *Staphylococcus aureus* and *Coagulase negative Staphylococci*.[2].

Among the gram negative bacteria causing CRI, the predominant isolate was *Acinetobacter baumannii* (9%). 8% *Pseudomonas aeruginosa*, 4.5% *E.coli*, 3.4% *Klebsiella oxytoca*, 2.2% *Klebsiella pneumoniae* followed by *Proteus mirabilis* and *Proteus vulgaris* 2.25% each.[Table 14]. The common bacteria isolated from catheter blood were MRSA(33.3%), *Pseudomonas aeruginosa*(16.6%) and *Acinetobacter baumannii*(50%).

The predominant organism isolated in urine with bacteriuria>10^5 cfu/ml was *E. coli* (72.7%) followed by (27.2%) *S.epidermidis*. In patients with respiratory infection the bacteria isolated from sputum was *Klebsiella pneumoniae*(50%) and MSSA(50%). Among the 12 swabs cultured from infected site MSSA and *E.coli* were isolated in each 25%. Of the 9 samples of catheter blood drawn for culture the same organism were isolated in corresponding peripheral blood culture also that MRSA(33.3%), *Pseudomonas aeruginosa, Acinetobacter baumannii* in 16.7% and 50% cases respectively. [Table 14]

Gram positive organisms were predominantly isolated in CRBSI namely *S.aureus* MRSA (31.4%), MSSA(8.5%), *S.epidermidis* (8.5%)and *S.schleiferi* (5.7%). Among the gram negative isolated from catheter related blood stream infection (14.2%) due to *Pseudomonas aeruginisa* ,(11.4%) *Acinetobacter baumannii* followed by *E.coli* (8.5%),*Klebsiella oxytoca*(5.7%), *Klebsiella pneumoniae* (2.8%) and *Proteus mirabilis* (2.2%). Our study correlates with the study of Pooja *et al*., that *E.coli* as the predominant gram negative bacteria[2]. Anil described that in his study 12.8% of CRBSI is due to *Acinetobacter baumannii, E.coli* (10.4%) *Pseudomonas aeruginosa* (2.3-15.2%), *Klebsiella pnemoniae*(6.4%).[23]However Ratnaja *et al* in reported (2-15%) *Pseudomonas aeruginosa,* (13%) *Acinetobacter* species in his study. In contrast to his study CRBSI due to Gram negative bacteria was isolated lesser (15%) than gram positive cocci. [Table15]

Ratnaja *et al*.[4] described (70.7%) of CRBSI was associated with gram positive organisms,(17.9%) by gram negative bacteria, (9.8%) with mixed gram positive and gram negative bacteria and MRSA(6-29%). Lynn Johnson *et al.* showed that the predominant bacterial etiological agent of CRBSI was *Staphylococcus aureus* and Coagulase negative *Staphylococci* [10]. Anil *et al*., also stated that 52-70% of CRBSI was due to gram positive cocci of that *Staphylococcus aureus* contributed (21.9-60%), *Staphylococcus epidermidis* (8.8-12%) and MRSA (6-8%) [23].Ramanathan *et al*, stated 26.7% of infection was due to MRSA [56]. Our study correlates with the study of Abdul Raguman *et al*. stated that (77%) of the CRBSI was caused by gram positive bacteria. [76], Hoen *et al* reported (68%) of CRBSI was due to gram positive organisms. [77]

In the present study polymicrobial infection was found commonly in CKD patients. *S.aureus* and *Candida albicans* were the predominant isolates in the polymicrobial growth (11.4%) followed by *Staphylococcus aureus* and *Klebsiella oxytoca*. Our study was similar to the study of Ratnaja *et al* [4], and Anil *et al* who reported that 16-20% polymicrobial growth was identified in patients with CRBSI. [Table 16]

Among the 35 cases of CRBSI (11.4%) cases developed hypotension and (5.7%) septic thrombophlebitis . The mortality rate was noted in (2.8%) of cases with CRBSI.[Table17].

A total of 12% fungal infections were noted in our study. Among the yeast like fungi isolated from CRBSI (5.6%) *Candida albicans, Candida parapsilosis* and *Candida tropicalis* were isolated from both catheter and blood. (11.6%) *Candida albicans* and *Aspergillus flavus*, (5.6%) of *Candida parapsilosis* and *Candida tropicalis*, and *Aspergillus fumigatus* were isolated only from catheter . Ratnaja *et al* reported 41% higher risk of CRBSI and Abbot *et al* reported that 79% of *Candida* infection occurs in early hemodialysis especially in patients with diabetes mellitus and anaemia.[94] Our study is similar to the study of Ratnaja *et al*[4], Hoen *et al* and Gandhi *et al*[13] in isolating fungal infections. Ramanathan *et al* reported (16%)of *Candida albicans* were isolated from central venous catheters in patients on HD.[56] Bernad *et al* [89], Monina *et al* [90] reported in 1.7% of *Candida albicans* infections in central venous catheters.[Table 18] (16.6%) *Candida tropicalis*, (22.2%) *Candida albicans* and (5.6%) of *Candida parapsilosis* were isolated from urine samples.

Parasitic infection was noted in 8.6%. The predominant parasite was *Entamoeba histolytica* (41.6%), *Giardia lamblia*(30.7%), *Cryptosporidium parvum*(15.3%) of cases and *Microsporidia*(7.6%) cases. .[Table 19] No malarial parasites were detected in the study group. Parasitic infections were noted in patients who were in an immunosuppressed state, diabetics and elderly age group. Seyrafian *et al* stated that 43.9%of HD patients infected with intestinal parasitic infection. Our study correlates with their study in detecting the parasitic infection in patients on HD. Kulik *et al* [14] and Seyrafian *et al*[75] stated the prevalence of *Blastocystis spp*(18%) was commonly noted in patients with renal impairment. *Endolimax nana*(14%), *Cryptosporidium parvum*(4-4.7%) and *Entamoeba coli* (4%) and (1.2%)*Giardia* were noted in patients with high uremic state. He stated that high uremic state and altered immune mechanism, neutropenia in HD patients promotes the parasitic infection and worsen the state of aneamia and aggravates the state of immunosuppression. Our study correlates with the study of Seyrafian *et al* in demonstrating the parasites in patients with renal impairment.

Anti microbial susceptibility testing was done as per CLSI guidelines. Among the Gram positive isolates *Staphylococcus aureus* and *Coagulase negative Staphylococci* showed 100% sensitivity to Amikacin and Amoxycillin clavulinic acid. A total of 37 isolates of Methicillin resistant *Staphylococcus aureus* all were sensitive to Vancomycin within the sensitive range of MIC. [Table 20,23]

Among the Enterobacteriaceae isolates screened for ESBL production 33.3% *Proteus mirabilis*, 37.5% of *Escherichia coli,* 20% of *Klebsiella pneumoniae*, 50% of *Proteus vulgaris* and 14.2% of *Klebsiella*

oxytoca were confirmed by Double disc synergy test. All these isolates showed 100% sensitivity to Imipenem and Cefoperazone sulbactum.

Among gram negative non fermenters *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* showed 100% sensitivity to Imipenem and Cefoperazone sulbactum. Among gram negative bacilli, production of extended spectrum β lactamaces was the mechanism of resistance in them. None of the isolates were found to be MBL producers and Amp C producers. In our study majority of the gram positive and gram negative organisms showed 100% sensitive to Amikacin.[Table 21,22 24]

Antifungal susceptibility testing was done by microdilution method as per the CLSI guidelines. *Aspergillus flavus* and *Aspergillus fumigatus* were sensitive to Amphotericin B, Voriconazole, Itraconazole within the MIC range. All the *Candida* isolates were sensitive to Fluconazole, Amphotericin B within the susceptibility range. None of the organisms isolated in our study had multi drug resistance.[Table 25,26]

SUMMARY

This study was conducted in the Institute of Microbiology, in association with Department of Nephrology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai from September 2011 to October 2012.

- A total number of 150 cases were included in the study after satisfying the inclusion and exclusion criteria.
- Out of the 150 patients, 78 males (52%) and 72 females (48%) were included in the study with the male predominance. Majority of the patients (67.4%) in the study group belonged to the age group of 21-30 years.
- Chronic kidney disease (70.7%) was the predominant Nephrological lesion for which hemodialysis was indicated.
- Diabetes mellitus (35%) was the most common underlying cause for hemodialysis among the chronic kidney disease, acute diarrheal disease (50%) was the predominant cause for HD among acute kidney injury patient.
- 58.3% and 23.3% of aerobic bacteria was isolated from catheter tip and blood respectively. 4.6% and 2% of fungi were isolated from catheter tip and blood respectively
- Gram stain demonstrated gram positive organism in 18% of cases. Gram negative organisms in 7.4% cases
- Among the 150 catheters processed 58.7% culture positivity was observed by roll plate method and 47.4%, 18.7% by vortex and segment washing method respectively.

- Colony count of $>10^2$ was noted in 58.7% of catheter and 23.3% peripheral blood by quantitative culture.
- 35.3% cases had catheter related infection without positive blood culture and categorized as catheter related infection(CRI). 23.3% cases with positive blood culture along with catheter were categorized as catheter related blood stream infection(CRBSI).
- CRBSI was noted commonly in the age group of 51-60 years (33.3%).
- Maximum number of catheters were inserted through internal jugular vein and CRBSI was noted in 20.8% of IJV catheters. But femoral catheterization had a higher risk of developing blood stream infection.
- Patients who have undergone hemodialysis more than 10 times showed maximum risk of developing CRBSI.
- 51.4% of catheter related blood stream infection was observed in patients with catheter in place for more than 15 days followed by 34.3% between 11-15 days.
- Diabetes mellitus was the commonest risk factor found in patient with CRBSI 94.2% followed by anaemia.
- 31.4% of MRSA was isolated from peripheral venous blood and 27.2% from catheter tip. Among the gram negative organism *Pseudomonas aeruginosa* was the predominantly isolated in catheter tip and peripheral venous blood 7%, 14.2% respectively
- Gram positive organisms (31.4%) MRSA was the predominant isolate in CRBSI. *Pseudomonas aeruginosa* (14.2%) was the predominant gram negative organism isolated in CRBSI.

- A total of 6 cases had poly microbial growth. *Staphylococcus aureus* and *Candida* species were isolated in 11.4% CRBSI
- Among the 35 cases of CRBSI 11.4% developed hypotension and 5.7% septic thrombophlebitis and mortality was noted in 2.8% cases.
- Out of the 18(12%) isolates of fungi *C.albicans, C.tropicalis, C.parapsilosis* constituted 5.6%.
- *Entamoeba histolytica* was predominantly seen in 46.1%
- *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus schleiferi* and *Staphylococcus warneri* showed 100% sensitive to Amikacin. 22.7%, 13.1%, 33.3% of MRSA were detected in catheter tip, peripheral venous blood and catheter blood respectively.
- Majority of the gram negative organisms were sensitive to Amikacin
- All the enterobacteriaceae isolates were sensitive to Cefoperazone and sulbactuam.
- 33.3% of *Proteus mirabilis*, 37.5% of *E.coli*, 16.6% *Klebsiella pneumoniae*, 50% of *Proteus vulgaris* and 14.2% *Klebsiella oxytoca* were ESBL producers.
- All the Methicillin Resistant *Staphylococcus aureus* were sensitive to vancomycin.
- All the *Candida* isolates were 100% sensitive to Fluconazole and Amphotericin B.
- *Aspergillus flavus* and *Aspergillus fumigatus* showed 100% sensitive to Amphotericin B, Itraconazole, Voriconazole.

CONCLUSION

This study was conducted in the Institute of Microbiology, in association with Department of Nephrology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai from September 2011 to October 2012.

Majority of the patients (67.4%) in the study group belonged to the age group of 21-30 years.

Catheter related infection was found in 53 (35.3%) of cases.

Catheter related blood stream infection was found in 35 cases (23.3%)

Fungal infection was seen in12% of patients who underwent hemodialysis.

Gram stain method provides rapid identification of infection in catheter.

Site, duration and number of catheterization play a major role in CRBSI and complications.

Negative Bacterial culture was seen in 62 cases (41.4%)

Parasitic infection was noted in 8.6% of patients who underwent hemodialysis

Gram stain method is complementary to roll plate method and indicates rapid identification of infection in catheter.

Semi quantitative roll plate method is superior to vortex and segment washing methods.

Quantitative culture of catheter tip and peripheral venous blood appears to be good approach in detecting catheter related blood stream infection.

Vortex and Endoluminal technique takes a longer time and requires technical expertisation.

Predominant organism causing blood stream infection was *Staphylococcus aureus* 31.4%, followed by *S. epidermidis*, 8.5%

All the MRSA were sensitive to vancomycin with the MIC less than 2µg/ml

Among the Gram negative bacteria *Pseudomonas aeruginosa*-14.2% and *Acinetobacter baumannii*-11.4% were the causatives for CRBSI.

50% of *Proteus vulgaris*, 33.3% of P. mirabilis and 37.5% of *Escherichia coli* were Betalactamase producers.

All gram positive and gram negative bacteria were sensitive to Amikacin.

Hypotension was the most common complication in patients with CRBSI.

Apart from diabetes mellitus and anaemia, history of infection in the previous dialysis and the number of dialysis were the common predisposing factors for CRBSI.

Following identification of *Acinetobacter baumannii* in catheter blood for three patients the catheters were removed and patients recovered and treated effectively.

None of the organism isolated in this study had multi drug resistance.

PREVENTION

Risk of Catheter Related Blood Stream Infection can be greatly reduced by strict aseptic technique.

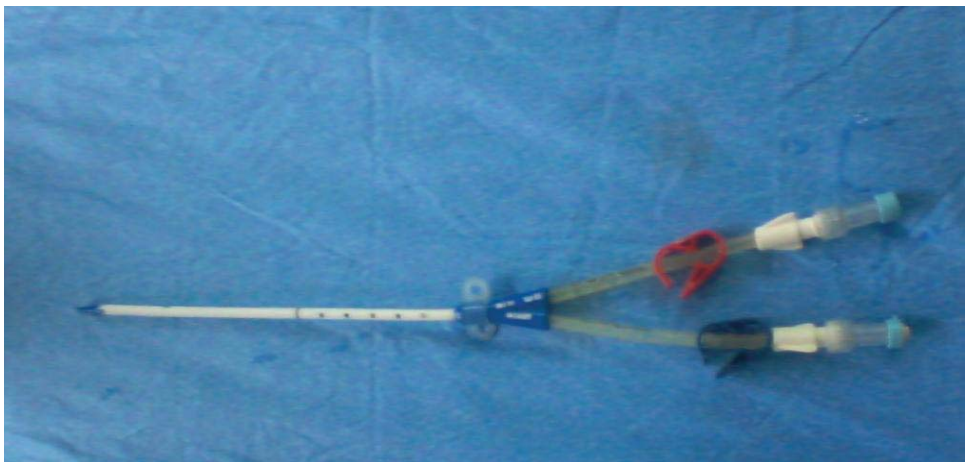
Strict hand washing should be practiced.

Changing of catheters with sterile precaution is mandatory.

Health education regarding taking care of catheter should be done.

Appropriate antibiotic therapy to prevent drug resistance is one of the important therapeutic approach in prevention of CRBSI.

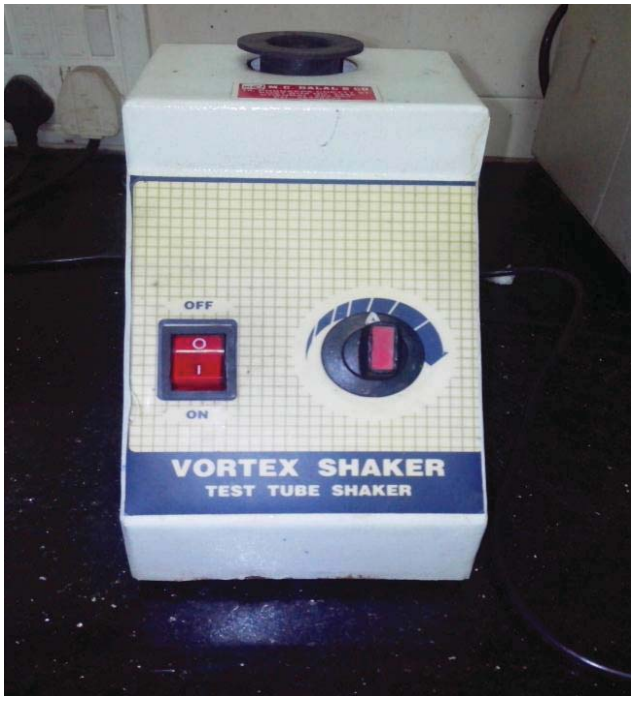
Double lumen Central Venous catheter



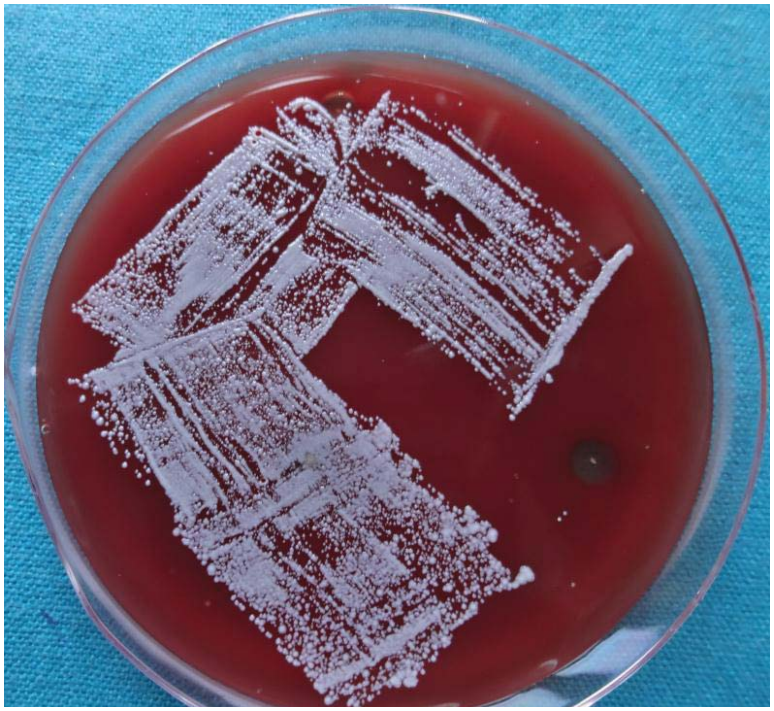
DOUBLE LUMEN CATHETER IN SITU

HEMODIALYSIS MACHINE

VORTEX SHAKER



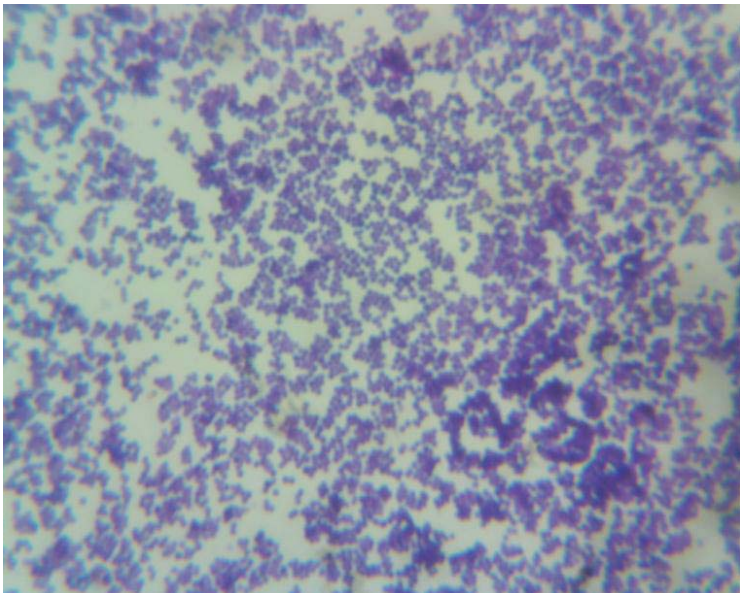
ROLE PLATE METHOD ON BLOOD AGAR PLATE



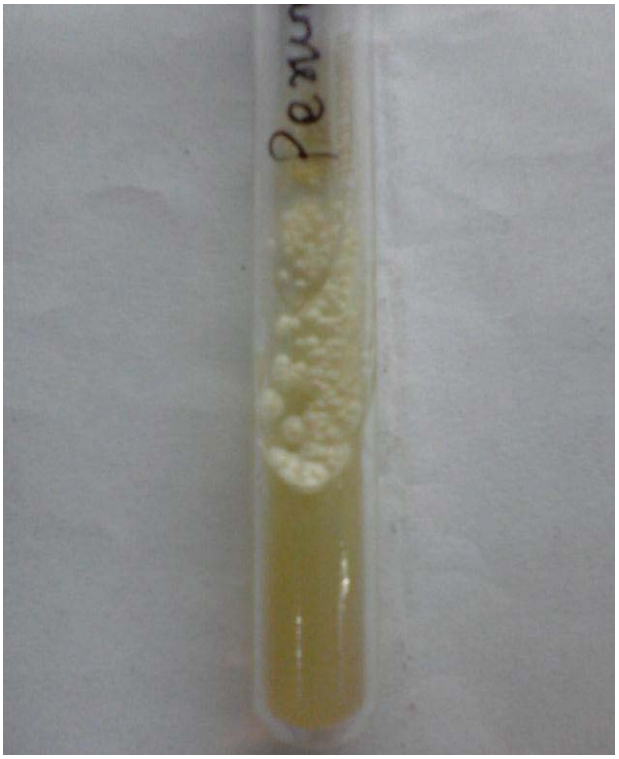
BLOOD AGAR PLATE

COLONY COUNT ON BAP

GRAM STAIN

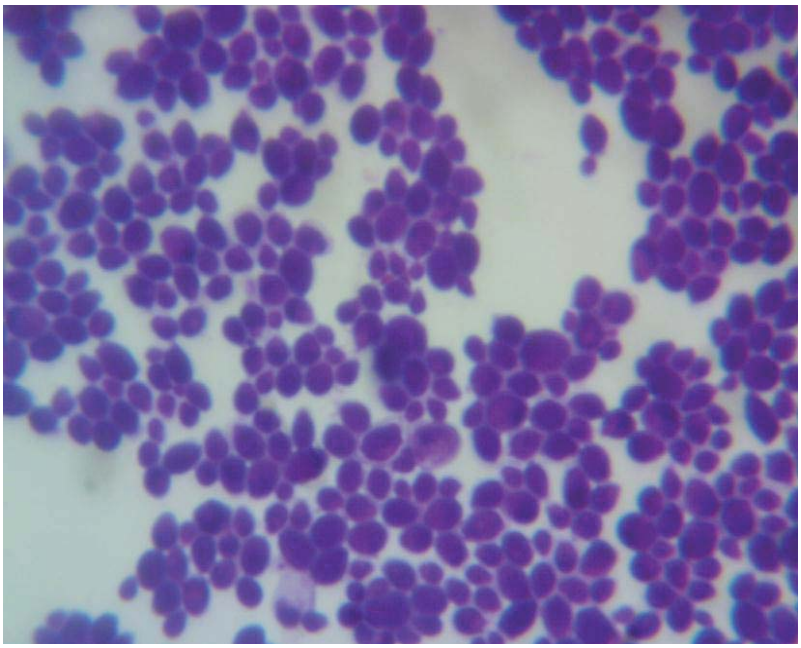


YEAST LIKE FUNGI ON - SDA

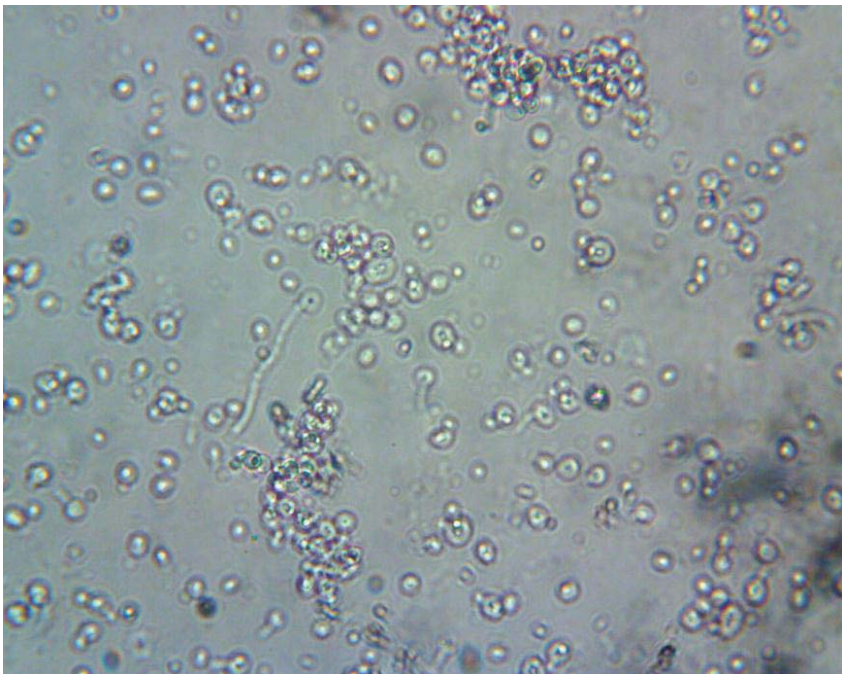


CHROME AGAR- CANDIDA SPECIATION

YEAST LIKE FUNGI

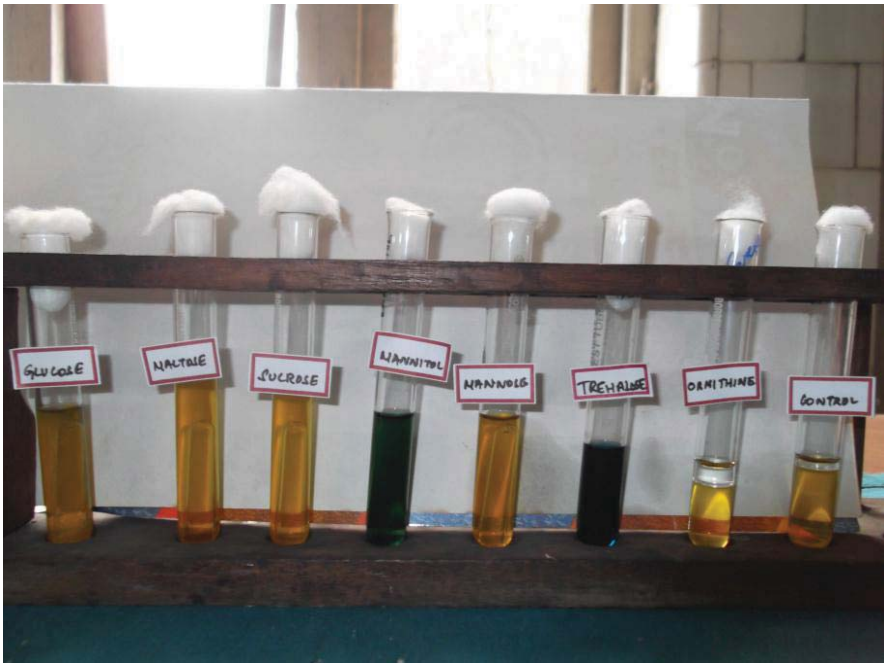


GERM TUBE TEST



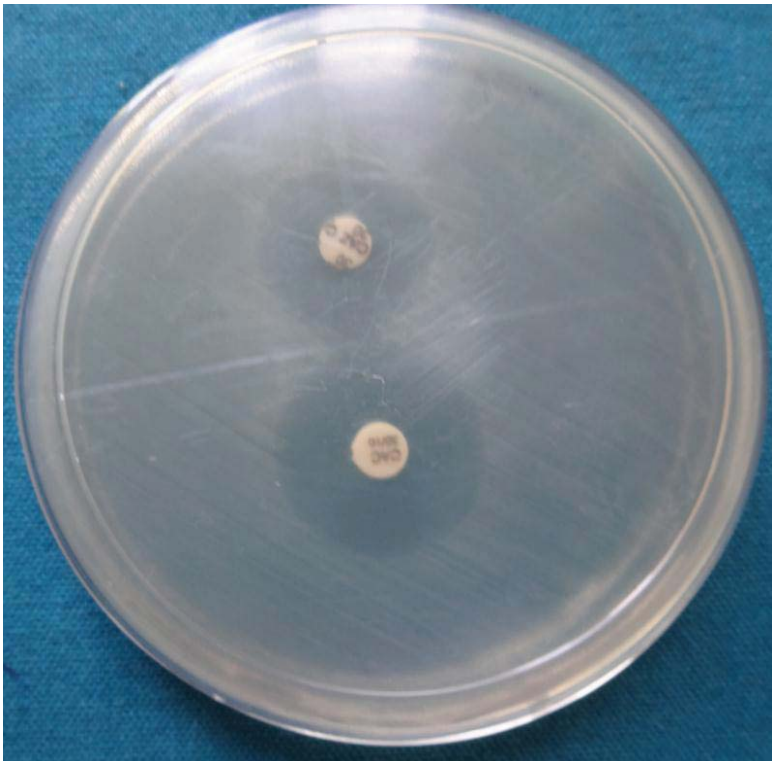
PHOSPHATASE TEST- SPECIATION OF COAGULASE NEGATIVE STAPHYLOCOCCI

BIOCHEMICAL REACTIONS OF S.EPIDERMIDIS



DOUBLE DISK DIFFUSION SYNERGY TEST FOR ESBL

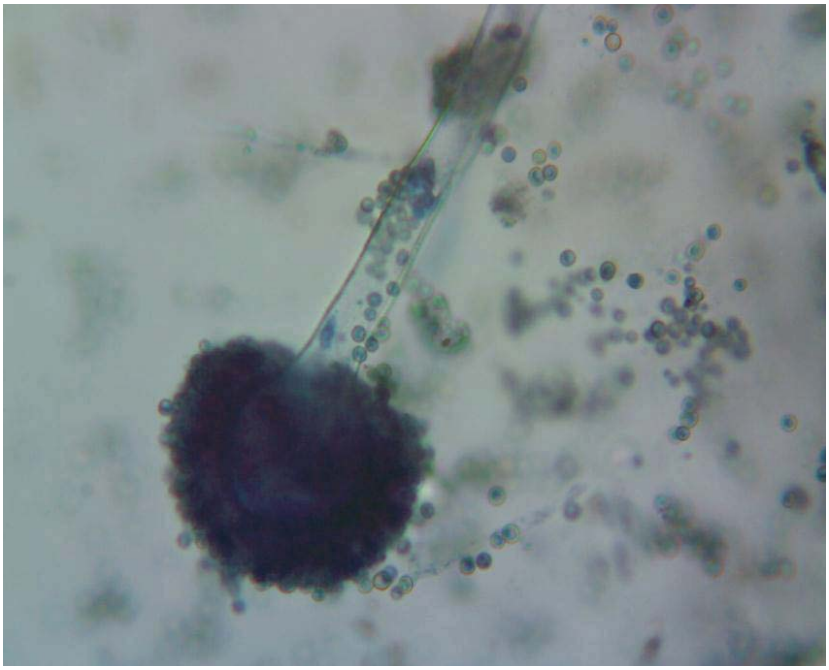
PHENOTYPIC CONFORMATORY DOUBLE DISK TEST



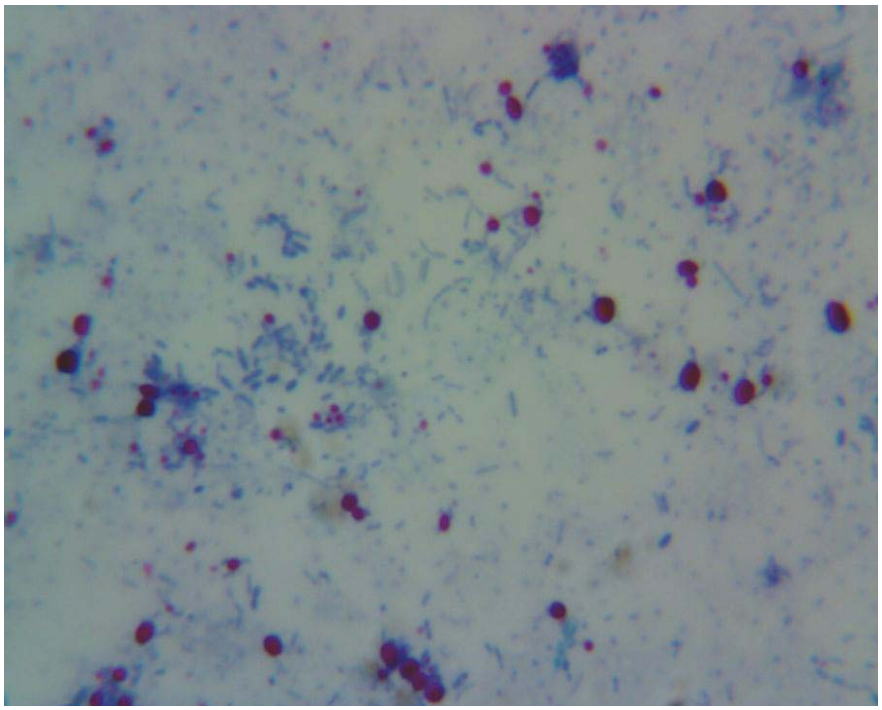
MIC OF VANCOMYCIN

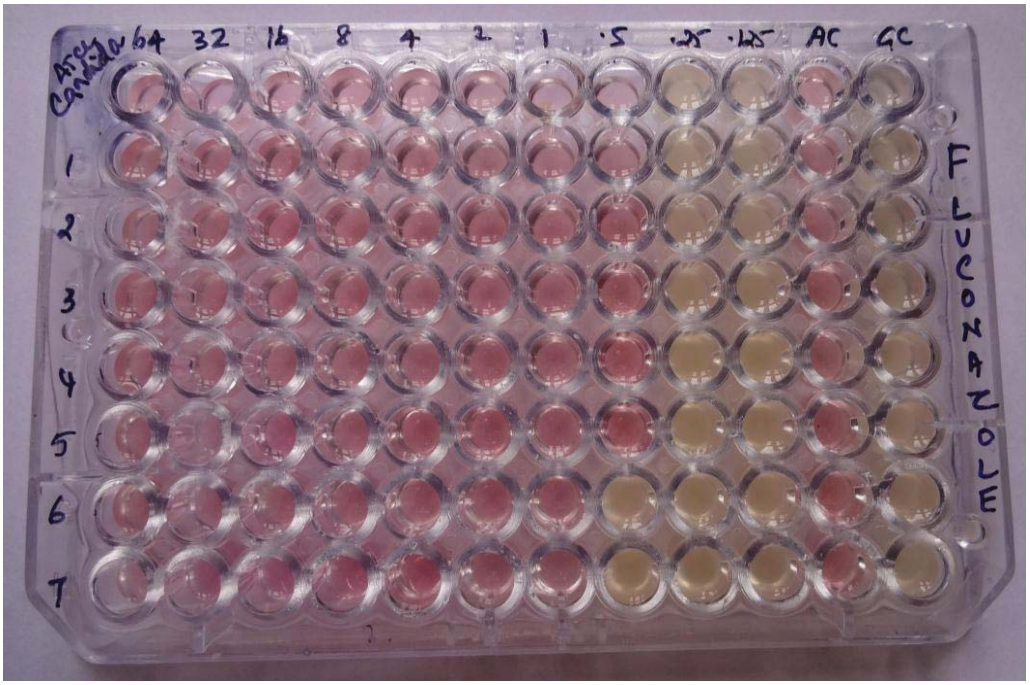
E-TEST FOR ESBL

LPCB - ASPERGILLUS FLAVUS

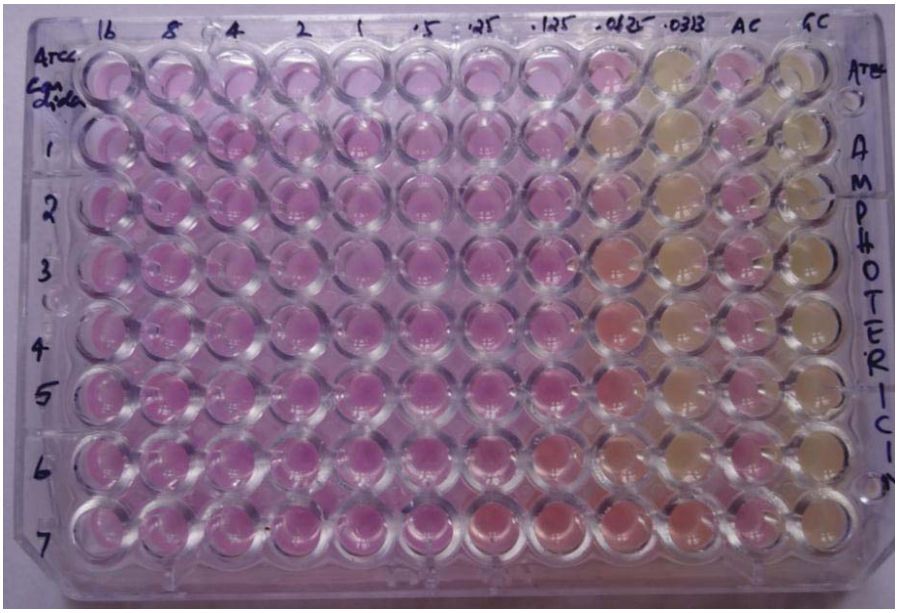


MODIFIED ACID FAST STAINING - CRYPTOSPORIDIUM PARVUM





MIC OF CANDIDA



BIBLIOGRAPHY

1. Kinchen KS, Sadler J, Fink N. The timing of specialist evolution in chronic kidney disease and mortality. *Ann Intern Med* 137:479-486, 2002.

2. Pooja Gupta, Reena Set, Kalpana Mehta, Jayanthi Shasthri. Incidence of Bactermia Associated with central venous catheter in Patient on Hemodialysis, *International Journal of Pharmacy and Pharmaceutical sciences*. Vol 3; Issue 3, 2011; ISSN-075-1491

3. Washington Winn, Stephen Allen, William Janda, Elmer koneman, Gary Procop, Paul Schrekenberger, Gail Woods in Koneman's Colour atlas and textbook of Diagnostic Microbiology, Sixth edition Chapter 2, p 100-2. Lippincott Williams and Wilkins.

4. Ratnaja Katneni and S.Susan Hedayati, Central venous catheter-related bacteremia in chronic hemodialysis patients: epidemiology and evidence – based management may 2007, Vol 3; No.5

5. Daiane Patricia Cais, Ruth naralia Teresa Turrini, Tania Mara Varejao Strabelli, Infections in patients submitted to hemodialysis: systematic revi ew, Rev Bras Ter Intensiva, 2009; 21(3):269-275

6. Tokars JI *et al*.(2001) A prospective study of vascular access infections at sevan outpatient hemodialysis enters. *Am J Kidney* Dis 37: 1232-40

7. Mandell, Douglas and Bennett. Bacteremia due to percutaneous intravascular device chapter 281 in Textbook of Infectious Disease by Mandell.7th edition

8. Yardena Siegnman-IGRA, Anne M. Anglim, David E, Shapiro, Karim A, Adai, Barbara A. Stain and Barry M.Farr, Diagnosis of vascular Catheter – Related Bloodstream Infection: a Meta – Analysis, *Journal of Clinical Microbiology*, Apr.1997, p.28-936

9. United states Renal Data System 1999 Annual Data Report Part IX Hospitalization in ESRD Am Kidney Dis 1999;34;114-23.

10. B Lynn Johnston, MD and John MConly MD, Central Venous catheter- associated bloodstream infection n hemodialysis patients: Another patient safety bundle? *Can J infect Dis Med Microbiol*. 2006 Mar-Apr, 17(2):

11. Robert w Schrier, Carl W Gottschalk Diseases of Kidney Vol-II, Section XIII

12. Chang CF, Kuo BIT, Chen TL, Yang WC, Lee SD, Lin CC. Infective endocarditis in maintenance hemodialysis patient Fifteen years' experience in one medical center. J Nephrol 2004;17:228-35

13. BV Gandhi, MM Bahadur, H Dodeja, V Aggrwal, A Tamba, M Mali, Systemic fungal infections, *Journal of Post Graduation Medicine 2005,* Vol: 51, Issue:5, Page 32-36

14. Rose Anne Kulik, Dina Lucia, Morasis Falavigna, Leticia Nishi and Silvana Marques Araujo, Blastocystis sp. and other Intestinal Parasites in Hemodialysis Patient, *The Brazillian Journal* of Infectious Disease, 2008;12(4) 338-341

15. Centers for Disease and prevention National nosocomial infections surveillance (NNIS) report, data summary from October 1986-April 1997, issue May 1997. *Am J Infect Control 1997*;25;277-87

16. Kievens RM,Edward JR,*et al* .Establishing health care and deaths in U.S hospitals, 2002.Public health reports 2007;122:160-166

17. William L.Henrich, Types of Vascular Access Chapter III in Principles and practice of Dialysis.

18. Stone H.H., Kolb L.D., Currie CA, Geheber CE 1974, Candida Sepsis. Pathogenesis and Principles of treatment, Annals of Surgery Vol-179, Page No: 697.

19. David L Veenstra, Sanjay Saint, Sean D Sullivan 1999. Cost effectiveness of Antiseptic Impregnated central Venous Catheter for the prevention of Catheter related blood stream infection. *Journal of American Medical Associa* -282 No. 6, Page: 554-460

20. Richard M. Stillman, Fawzi Soliman, Luis Garcia 177, Etiology of Catheter Associated Sepsis. Archives of Surgery Vol-112 Page: 1497-1499

21. Luc Michel, John GMC Michan, Louis Bachry 1979, Microbial Colonisation of Indwelling Central venous catheters: statically evaluation of Potential contaminating factors, *Journal of surgery* Vol-137.

22. Harlan H. Stone, Laura Kolb, Camila Curice A, Carol E Geheber 1973 Candida Sepsis, Pathogenesis and Principles of treatment Annual Meeting of the Southern Surgical Association.

23. Anil K, Saxena, Bodb R, Panbotra, Haemodialysis catheter- related bloodstream infections: current treatment options and strategies for prevention, *Review article, Swiss med wkly* 2005; 135:127-138

24. Moist, L.M, Tryeski,and Lok, C.E. Increased hempdialysis catheter use in Canada and mortality risk: data from the Canadian organ transplant registry.2001-2004. 2008.*Clin J.AM Soc.Nephro.* 3, 1726-32

25. Saxena AK, PanhotraBR, Naguib M, *et al*, Septicemia in hemodialysis; A focus on bacterial flora and antibiotic access salvage. Saudi J Kidney Dis Transplant 2002; 13:29-34.

26. Harsha V Patil, Virendra C Patil, MN Ramteerthkar, RD Kulkarni, Central Venous Catheter – related blood stream infections in the intensive care unit, *Indian Journal of Critical Care Medicine,* 2011, Volume:15, Issue:4, Page:213-223.

27. Emilio Bouza, Neisa Alvarado, Luis Alcala, Matilde Sanchez-Conde, Maria Jesus Perez, Patricia Munoz, Pablo Martin-Rabadan

and Marta Rodriguez-Creixems, A prospective, Randomized and comparative study of 3 different Methods for the diagnosis of Intravascular catheter colonization. 2005; 40:1096-100

28. Issam I Raad and Gerald P.Bodey, Infectious Complications of Indwelling Vascular Catheters, *Clinical Infectious Disease* 1992, 15: 197-210.

29. Catheter Related Bacteremia, due Roseomonas gilardil 1997 *Journal of Clinical Microbiology* Vol-35, No.10, Page No. 2712.

30. Susan Varghese, Padmaja P. Sudha P 1998, Central Venous Catheter related infections. *Journal of Communicable Disease* Vol-146, No.4 page 49 to 482.

31. Betty Forbes, Daniel Sahm, Alice Weissfeld. Bailey & Scott's Diagnostic Microbiology. Chapter 52- Blood Stream Infections 780-789. Mosby Elsevier publishers

32. Federico Bozetti, Giovanni Terno, Edgasrda Camerini 1981,Pathogenesis and predictability of central venous catheter sepsis. Surgery vol 91 No.4 Page 383-389.

33. Patti Jm, Allen BL, McGavin MJ, Hook M.MSCRAMM-mediated adherence of micro organisms to host tissues. Annu Rev Microbiol 1994;118:199-205

34. Maki DG, Weise CE, Sarafin HW, A semiquantitative culture method for identifying intravenous – catheter – related infection.

culture method for identifying intravenous – catheter – related infection. N Engl J Med. 1977; 296 (23): 1305-9.

35. Georg Peter, Romano Locci and Gerhard Pulverer 1982. Adherence and grwothof coagulate Negative staphylococci on surfaces of intravenous catheter. *The Journal of infectious disease* Vol-146, No.4 Page No:479 to 482.

36. Susan Varghese, Padmaja P. Sudha P 1998, Central Venous Catheter related infections. *Journal of Communicable Disease* Vol-131, No.1, page 1 -4.

37. De Andrade D, Ferreira V. Central Venous access for haemodilysis: Prospective evaluation of possible complications. *J.Clin Nurse.* 2007; 16 (2): 414-8

38. Angella Goetz. Victor L, Yu., James E Hanchett 1983. Pseudomonas stustzeri Bactermia associated with hemodialysis. Archives of Internal Medicine Vol: 143 Page 1909-1912.

39. Kieren A. Mary, Daniel J, Sexton, Pater J Conlon, G. Ralph Corey 1997. Catheter Related Bacteremia and Outcome of Attempted catheter salvage in patients undergoing hemodialysis. Annals of internal medicine Vol-127, Page 275-280.

40. Stephen J Uman, Caroline E Johnson, aregory J. Beirne 1977 Pseudornonas. aeruginosa Bacterernia in a Dialysis init. *American Journal of Medicine* Vol-62 page 667-67 1.

41. Theresa M. Haslctt, Henry D Isenberg, Eileen Hilton 1988. Microbiology of Indwelling Central Intravascular Catheters. *Journal of clinical Microbiology* Vol-26 No.4 page 696-701.

42. Jade Zufferey, Bernadette Rime, Patrick Francioli 1988. Simple Method for Rapid diagnosis of Catheter Associated Infection by Direct Acridine Orange staining of Catheter tips. *Journal of clinical Microbiology* Vol-26 No.2 Page 175-177.

43. Malamateriia Arvantidou, Sophia Spaia, Christos katsinos, Panayotis Pangidis. 1998 Microbiological quality of water and dialysate in all hemodialysis centres of Greece. Nephrology, Dialysis, Transplantation Vol-13 page 949-954.

44. Elise M Jochimsen, Charles Frenette, Monique Delorme, Mathew Arduino, Sonia Aguero 1998. A cluster of Blood stream infections and pyrogenic reactions among hemodialysis patients traced to Dialysis Machine waste Handling option units American *Journal of Nephrology* Vol-18 Page 485-489.

45. Dopirak M. Hill C, Oleksiw M., Dumigan D, Arvai J, English E, *et al*., Surveillance of hemodialysis-associated primary blood stream infections: the experience of ten hospital-based centers. Infect control hosp Epidemiol 2002; 23 (12):721-4.

46. CDC: Invasive methicillin-resistant Staphylococcus aureus infections among dialysis patients- united states, 2005. SMMWR Recomm Rep 56:197, 2007

47. Jerome I, Tokars, MD, MPH, New national surveillance system for hemodialysis – associated infections: Initial results, *American Journal of Infection control* 2002; 30:288-95.

48. Diane Benezera, Timothy E Kiehn, Jonathan W.M., Gold, Arthur E 1988. Prospective study of Infections in Indwelling Central Venous Catheters Using quantitative Blood cultures. *American Journal of Medicine* Vol-85 age 495-498.

49. Edward J Wing, Carl W Norden, Richard K Shadduck, 1979. Use of quantitative bacteriologic techniques to diagnose catheter related sepsis. Archieves of internal Medicine vol-139 page 482-483.

50. Francois Blot, Eric Shmidt, Gerard Nitenbcrg, Cynic Tancrede 1998. Earlier positivity of Central Venous Versus Peripheral blood cultures in highly predictive of catheter related sepsis *Journal of Clinical Microbiology* Vol-36 No.4 page 105-109.

51. Car1os V Paya, Luis Guerra, H. Michael Mars, Michael B Farnell 1989. Limited usefulness of quantitative cultures of blood drawn through the device 1o' diagnosis of Intravascular Device related bactermia *Journal of Clinical Mirrobio1ogy* Vol-27 No.7 page 1431-1433.

52. Raymond Vanholder, Bernard Canaud, Richard Fluck, Michel Jadout, Laura Labriola, Anna Marti – Monros, Jan Tordoir and Wim Vam Biesen Catheter-related Blood stream infection (CRBSI): a European view, 2010, 1 of 4, doi: 10.1093.

53. Leonard A. Mermel, Michael Allon, Emilio Bouza, Donald E. Carven, Patricia Flynn, Naomi P.O.Grady, Issam I. Raad, Bart J.A. Rijnders, Robert J.Sheretz and David K.Warren, Clinical Practice Guidelines for the Diagnosis and Management of Intravascular Catheter – Related infection: 2009 update by the Infectious Disease Society of America, CID 2009:49:1-45

54. Serkan Oncu, Serhan Sakarya., Central Venous Catheter – Related Infections: An overview with Special Emphasis on diagnosis, Prevention and Management, *The Internet Journal of Anesthesiolgy ISSN* : 1092-406X

55. Rello, J., J.M.Gatell, J.Almiral, L.M. Campistol, J.Gonzatez and J.puigde la Bellacasa. 1989, Evaluation of culture techniques for identification of catheter- related infection in hemodialysis patients. Eur. J.Clin.Microbiol Infect Disc 8:620-622

56. Ramanathan Paramewaran, jatan B. Shrchan, Muralidhar Varma D, Chiranjay, Mukhopadhyay, Sudha Vidyasagar Intravascular catheter – related infections in an Indian tertiary care hospital. J Infect Dev Ctries 2011; 5 (6):452-458.

57. Procedure for semi-quantitative cultures of central lines, Developed by MHA-KICU Infectious Disease Advisory Group – Final 06-2006

58. Jagadish Chander Text book of Medical Mycology, sixth edition. Appendix A, Fub=ngal Culture Media 509-513. Mehta publishers

59. D.R.Arora, Medical Parasitology,third edition,chapter 13 Page 235-236. CBS Publishers and Distributors.

60. Washington, C.Winn, Stephen, Allen, William, Janda, Elmer, Koneman, 6th Edition of Text Book of Microbiology, Page No 1159

61. National Committee for Clinical Laboratories Standards.disk diffusion : supplemental tables M100-S13.NCCLS, Wayen, PA, USA, 2003

62. Husam S. Khanfar, Khalid M. Bindayna Extended spectrum betalactamases (ESBL) in Escherichia coli and Klebsiella pneumoniae:trends in the hospital and community settings J Infect Dev Ctries 2009; 3(4):295-299.

63. Performance standards for antimicrobial susceptibility testing; 15th informational supplement (M100-S15) (2005) National Committee for Clinical Laboratory Standards, Wayne, Pa, USA, National Committee for Clinical Laboratory Standards.

64. Chaudhary U,Aggarwal R Extended spectrum b lactamases- An emerging threat to clinical therapeutics *Indian Journal of medical Microbiology* 2004; 22(2) 75-80

65. Singhal *et al*. Evaluation of methods for AmpC β lactamases in gram negative isolatesfrom tertiary care hospitals. *Indian journal medical microbiology* 2005 23(2) 120-124

66. Chaudhary *et al*: Imipenem-EDTA disk method for rapid identification of metallo-b-lactamase producing Gram-negative bacteria Indian J Med Res 127, April 2008, pp 406-407

67. Lee, Y S Lim, D Yong, J H Yum, and Y Chong. Evaluation of Hodge test and the Imipenem-EDTA Double Disc Synergy Test for differentiating Metallo-beta lactamase producing isolates of Pseudomonas spp.and Acinetobacter spp. *Journal of Clinical Microbiology,* Oct2003,p.4623-4629.

68. Clarence J. Fernandes, Lorna A. Fernandes and Peter Collignon Cefoxitin resistance as a surrogate marker for the detection of methicillin-resistant Staphylococcus aureus *Journal of Antimicrobial Chemotherapy* (2005) 55, 506–510

69. National Committee for Clinical Laboratories Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: Approved standard M7-A6. NCCLS. Wayen. P A.USA, 2003.

70. Dr. G.S.Vijaya kumar, Dr. Madhuri Kulkarani, Dr. Sumana.M.N., Dr.Tejashree.A *et al*. Hands on Workshop, Antimicrobial Susceptability Testing 21st &22nd August 2010. Department of Microbiology, JSS Medical College, Mysore.

71. Merrer J, De Jonghe B, Golliot F, Lefrant JY, Raffy B, Barre E, Rigaud JP, Casciani D, Misset B, Bosquet C, Outin H, Brun-Buisson C, Nitenberg G (2001) Complications of femoral and subclavian venous catheterization in critically ill patients JAMA 286:700-707.

72. Goetz AM, Wagener MM, Miller JM, Muder RR (1998) Risk of infection due to central venous catheters: effect of site of placement and catheter type. Infect control hosp Epidemiol 19:842-845.

73. Mermel LA, Allon M, BOuza E, Craven DE, Flynn P, O/'Grady NP, *et al*. Clinical practice guideli diagnosis and management of intravascular catheter – related infection: 2009 update by the infection disease society of America. Clin Infect Dis 2009; 49:1.

74. Eyes S, Brummitt C, Crossley K, Siegel R, Cerra F. Catheter related sepsis: Prospective randomiz three methods of longterm catheter maintenance. Crit Care Med 1990; 18:1073-9.

75. Seyrafian SH, Pestechian N, Kerdegari M, Yousefi HA, Bastani B, Prevalence rate of cytptosporidium infection in hemodialysis patients in Iran. hemodialysis International 2006;10:375-9.

76. Abdulrahman, S.L., Bokhary, H.A., Ladipo, GO (2002) A Prospective study of Hemodialysis associated infection. J Infect Chemother 8: (3) 242-246

77. Hoen, B., Agnes, P., Kessler, M. (1998) Epibacdial: Risk factors for Bacteremia in chronic Hemodialysis patients J am Soc Nephrol 9: 869-879.

78. Brun-Buisson C, Abrouk F, Legrand P, Huet Y, Larabi S, Rapin M. diagnosis of Central venous catheter – related sepsis: critical level of quantitative tip culture, Arch Intern Med 1987: 147:873-7

79. Cleri DJ, Corrado ML, Seligman SJ. Quantitative culture of intravenous catheter and other intravascular inserts. J Infect Dis 1980; 141; 781-6

80. Sheret RJ, Raad II, Belani A, Koo LC, Rand KH, Pickett DL. Three year experience with sonicated vascular catheter culture in a clinical microbiology laboratory. J Chin Microbiol 1990; 28:7-82.

81. Siegman – Igra Y, Anglim AM, Shapiro DE,Adal KA, Strain BA, Farr BM. Diagnosis of vascular catheter – related blood stream infection: a meta – analysis J Clin Microbiol 1997; 35:928-36.

82. Surendara kumar p., Venu G, Madhusudhana Rao, Balakrishnan N, Saravanan T, Sofia Rani A, Subba Rao T.M. Prevalence and Risk factors of Hepatitits C among Maintenance Hemodiaylsis Patients at a Teritiary – care Hospital in Coimbatore, India.

83. Chandra M, Khaja MN, Hussains MM, Poduri CD, Farees N, Habeeb MA, etal., Prevalence of hepatitis B and Hepatitis C viral infections in Indian patients with chronic renal failure. Intervirology 2004;47:44-51.

84. Redy GA, Dakishnamurthy KV, Neelaprasad P, Gangadhar T, Lakshmi V. Prevalence of HBV and HCV dual infection in patients on hemodialysis. Indian J Med Microbial 2005; 23:41-43

85. Roberts TL *et al* (2004) Relationship among catheter insertions, vascular access infections and anemia management in hemodialysis patients. Kidney Int 66:2429-2436.

86. Allon M (2004) Dialysis catheter- related bacteremia: treatment and prophylaxis. Am J Kidney Dis 44: 779-791

87. Nassar GM and Ayus JC (2001) infectious complication of the hemodialysis access. Kidney Int 60: 1-13

88. Mayhall, C.G.1992. Diagnosis and management of infections of implantable device used for prolonged venous access curr. *Clin. top infect Dis.* 12: 83-100.

89. Bernard Canaud. Heaemodialysis catheter related infection: time for action, *Nephrol Dial Transplant* (1999) 14:2288-2290

90. Monina Klevens.R, Jonathan R.Edwards, Mary L.Andrus, Kelly D.Peterson, Margaret A. Dudeck, Teresa C.Horan and the NHSN Participants in Outpatient Dialysis Surveillance. Dialysis Survelillance Report: National Health Care safety Network (NHSN)2006. Division of Health care quality promotion, centers for Disease control and prevention, seminars in Dialysis –Vol 21, No.1 2008; p.24-28

91. Kieren A, Mary,d ANIELj, Sexton, Pater J C onlon , G. Ralph Corey 1997. Catheter related Bacteremia and Outcome of Attempted Catheter salvage in patients Undergoing hemodialysis.Annals of Internal Medicine Vol 127 p275-280

92. Thomas Bregenzer, Dieter Conen, Pascal Sakmann, Adreas F., Widmer 1998. Is Routine Replacement of Peripheral Intravenous Catheters Necessary? Archieves of Internal Medicine Vol =- 158 Page 151-156

93. Susan Varghease,Padmaja, Sudha Central venous catheter related infections. *Journal of Communicable Disease*,V ol-131 (1)P1-4

94. Abott KC, Hypolite I,. Hospitalizations for fungal infections after initiation O: States. Nephron 2001;89:426-32

95. Powe, N.R., Jaar .B., Furth, S.I, Briggs, W (1995) septicemia in dialysis patients: incidence, Risk factors and prognosis kidney international 55:1081-1091

96. Jonathan Himmelfarb, MD, Hemodialysis Complications. American *Journal of Kidney Disease,* Vol45, No 6 (June) 2005: pp 1122-1131.

97. Marr KA *et al*. (1997) Catheter-related bacteremia and outcome of attempted catheter salvage in patients undergoing hemodialysis. *Ann Intern Med* 127:275-280

98. Peter Kite, Brian M. Dobbins, Mark H. Wilcox 1999. Rapid diagnosis of central venous catheter related Blood stream infection without catheter removal Lancet Vol-354 Page: 1504-1507

ABBREVIATIONS

AKI	-	Acute Kidney Injury
ARF	-	Acute Renal Failure
ATCC	-	American Type Culture Collection
AVF	-	Arterio Venous Fistula
BSI	-	Blood Stream Infection
CDC	-	Centers for Disease Control
CKD	-	Chronic Kidney Disease
CLSI	-	Clinical and Laboratory Standards Institute
CRBSI	-	Catheter Related Blood Stream Infection
CRI	-	Catheter Related Infection
CVC	-	Central Venous Catheter
ERBP	-	European Renal Best Practice
HD	-	Hemodialysis
HIPAC	-	Healthcare Infection control Practices Advisory Committee
IDSA	-	Infectious Disease Society of America
IJV	-	Internal Jugular Vein
IVC	-	Intravascular catheter
MHC	-	Major Histo Compatibility Complex
RRT	-	Renal Replacement Therapy

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI -3

Telephone No: 04425305301
Fax : 04425363970

CERTIFICATE OF APPROVAL

To
Dr. R. Synthia Selvakumari
PG in MD Microbiology
Madras Medical College, Chennai -3.

Dear Dr. R. Synthia Selvakumari

The Institutional Ethics Committee of Madras Medical College reviewed and discussed your application for approval of the proposal entitled " A study on Bacterial, fungal and Parasitic infections in patients on haemodialysis and their antimicrobial susceptibility pattern in a tertiary care hospital " No. 18102011.

The following members of Ethics Committee were present in the meeting held on 20.10.2011 conducted at Madras Medical College, Chennai -3.

1.	Prof. S.K. Rajan, MD	-- Chairperson
2.	Prof. A. Sundaram, MD Vice Principal , Madras Medical College, Chennai -3	-- Member Secretary
3.	Prof R. Nandhini, MD Director, Institute of Pharmacology, MMC, Ch-3	-- Member
4.	Prof. C. Rajendiran, MD Director , Institute of Internal Medicine, MMC, Ch-3	-- Member
5.	Thiru. A. Ulaganathan Administrative Officer, MMC, Chennai -3	-- Layperson
6.	Thiru. S. Govindasamy . BA.BL	-- Lawyer
7.	Tmt. Arnold Soulina MA	-- Social Scientist
8.	Prof. Shanta Ravishankar Prof of Neuropathology, M M C, Chennai -3	-- Member

We approve the proposal to be conducted in its presented form

Sd /. Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, any SAE occurring in the course of the study, any changes in the protocol and patient information / informed consent and asks to be provided a copy of the final report

Member Secretary, Ethics Committee

PROFORMA

Name : IP No :

Age : Ward :

Sex :

Occupation :

Address :

Date of Admission:

Presenting Complaints:

1. Fever 2.Chills 3. Head ache 4. Vomiting

5. Abdominal Pain 6. Diarrhea

Local Examination

1. Warmth 2. Redness 3.Swelling 4. Discharge

Risk Factors/ Demographic Profile of the patients:

Prior location before admission

Hypertension

Prior antibiotic therapy

Known retroviral disease

H/o.Blood transfusion

Hepatitis B

Hepatitis C

Steroid Therapy

Diabetes

H/o. Prior dialysis

H/o. Renal transplant or Renal biopsy

H/o. Infection during previous heemodialysis or hospitalization

Nature of renal disease

Duration of dialysis & catheterization

Laboratory evaluation:

Complete haemogram

Platelet Count

ESR

Blood Sugar: Fasting & Post Prandial

Urea

Creatinine

Urine Routine

USG Abdomen

MICROBIOLOGICAL INVESTIGATION

a. Microscopic examination

Gram Stain

Giemsa Stain

Wet Mount

Lactophenol cotton blue mount

Iodine mount

Potassium Hydroxide mount

Modified acid fast staining

b. Culture

Bacterial Culture

Fungal Culture

c. Antimicrobial susceptibility pattern

d. Antimicrobial susceptibility pattern

CONSENT FORM

STUDY TITLE :

A study on Bacterial, Fungal and Parasitic infections in patients on haemodialysis and their antimicrobial susceptibility pattern in a Tertiary Care Hospital

I ....., hereby give consent to participate in the study conducted by Dr.R.Synthia Selvakumari, Post graduate at Institute of Microbiology, Madras Medical College, Chennai and to use my personal clinical data and the result of investigations for the purpose of analysis and to study the bacterial, fungal, and parasitic isolates. I also consent to give my blood, urine, stool and sputum for further investigations. I also learn that there is no additional risk in this study. I also give my consent for my investigator to publish the data in any forum or journal

Signature of the Place Date

Health care provider

Name & Address:

Signature of the investigator:

Signature of the guide:

APPENDIX

A. STAINS AND REAGENTS

I. Gram staining

Methyl violet (2%)	l0g Methyl violet in 100ml absolute alcohol in 1litre of distilled water (primary stain)
Grams Iodine	l0g Iodine in 20g KI (fixative)
Acetone	Decolourising agent
Carbol fuchsin 1%	Secondary stain

II. Lactophenol cotton blue stain

Lactic acid	20 ml
Phenol	20ml
Cotton blue (dye)	0.5g
Glycerol	40ml
Distilled water	20ml.

III. 10% KOH

Potassium hydroxide	l0g
Glycerol l0ml	
Distilled water	80ml

LUGOLS IODINE

Pottasium Iodide	10 gm
Iodine	5 gm
Distilled Water	100 ml

B. MEDIA USED

1. Mac Conkey agar

Peptone	20g
Sodium taurocholate	5g
Distilled Water	1 ltr
Agar	20g
2% neutral red in 50% ethanol	3.5ml
10% lactose solution	100mI

Dissolve peptone and taurocholate in water by heating. Add agar and dissolve it in steamer. Adjust pH to 7.5. Add lactose and neutral red shake well and mix. Heat in free steam (100°C) for 1 hour, then autoclave at 115°C for 15 minutes.

2. Blood agar (5% sheep blood agar)

Peptone	l0g
NaCl	5g
Distilled water	1 Ltr
Agar	10g

Dissolve ingredients in distilled water by boiling, and add 5% sheep blood(sterile) at 55°C adjust pH to 7.4.

3. Chocolate agar

Sterile defibrinated blood	10 ml
Nutrient Agar (melted)	100 ml

When the temperature was about 75°C, sterile blood was added with constant agitation. After addition of blood, kept in water bath and heating was continued till the blood changed to chocolate colour. Cooled to about 50° C and poured about 15ml into petri dishes with sterile precaution.

4. Sabouraud's dextrose agar

Dextrose	40g
Peptone	l0g
Agar	20g
Distilled water	l000ml

pH = 5.5

5. Mueller- Hinton Agar

Beef infusion	300ml
Caesein hydrolysate	17.5g
Starch	1.5g
Agar	10g
Distilled water	1 ltr

pH = 7.4

Sterilise by autoclaving at 121ºC for 20 mins

C. MEDIA REQUIRED FOR BIOCHEMICAL IDENTIFICATION

1.Oxidase Reagent

Tetra methyl p-phenylene diamine dihyrochloride- 1% aqueous solution.

2.Catalase

3% hydrogen peroxide

3.Indole test

Kovac's reagent

Amyl or isoamyl alcohol	150ml
Para dimethyl amino benzaldehyde	10g
Concentrated hydrochloric acid	50ml

Dissolve the aldehyde in the alcohol and slowly add the acid. Prepare in small quantities and store in the refrigerator. Shake gently before use.

4.Christensen's Urease test medium

Peptone	1g
Sodium chloride	5g
Dipotassium hydrogen phosphate	2g
Phenol red	6ml
Agar	20g
Distilled water	1 ltr
10% sterile solution of glucose	l0ml
Sterile 20% urea solution	100ml

Sterilize the glucose and urea solutions by filtration. Prepare the basal medium without glucose and urea, adjust to pH 6.8-6.9 and sterilize by autoclaving in a flask at 121°C for 30 min. Cool to about 50°C, add the glucose & urea, and tube the medium as slopes.

5. Simmon's Citrate Medium

Koser's medium	1 ltr
Agar	20g
Bromothymol blue 0.2%	40ml

Dispense, autoclave at 121°C for 15 min and allow to set as slopes

6. Triple Sugar Iron medium

Beef extract	3g
Yeast extract	3g
Peptone	20g
Glucose	1g
Lactose	l0g
Sucrose	l0g

Ferric citrate	0.3g
Sodium chloride	5g
Sodum thiosulphate	0.3g
Agar	12g
Phenol red 0.2% solution	12ml
Distilled water	1 ltr

Heat to dissolve the solids, add the indicator solution, mix and tube. Sterilize at 121°C for 15 min and cool to form slopes with deep butts.

7. Glucose phosphate broth

Peptone	5g
Dipotassium hydrogen phosphate	5g
Water	1 ltr
Glucose 10% solution	50ml

Dissolve the peptone and phosphate and adjust the pH to 7.6. Filter dispense in 5ml amounts and sterilize at 121°C for 15min. Sterilize the glucose solution by filtration and add 0.25ml to each tube.

Methyl Red Reagent

Methyl Red	10mg
Ethyl alcohol	30ml
Distilled water	20ml

Voges Proskauer Reagent

Reagent A: Alpha naphthol	5g
Ethyl alcohol	100ml
Reagent B: Potassium hydroxide	40g
Distilled water	100ml

8. Peptone water fermentation test medium.

To the basal medium of peptone water, add sterilised sugars of 1% indicator bromothymol blue with Durham’s tube.

Basal medium peptone water

Sugar solutions:	
Sugar	1ml
Dislilled water	100ml
pH = 7.6.	

9. Mannitol motility medium

Agar	5g
Peptone	1g
Potassium nitrate	1g
Mannitol	2g
Phenol red indicator	
Distilled water	1000ml
pH 7.2	

10. Phenolphthalein diphosphate agar

Sterilize a 1% aqueous solution of sodium phenolphthalein diphosphate by filtration and store at 4ºC

Add 10ml of this solution to 1000ml melted nutrient agar cooled to 50ºC and pour plates

Grow the staphylococcus overnight at 37ºC on the medium

Invert the plate and pour a few drops of ammonia solution SG 0.88 into the lid

Read as positive a culture whose colonies turn bright pink within a few minutes. The colour soon fades.

11. Potassium nitrate broth

Potassium nitrate (KN03)	0.2gm
Peptone	5.0gm
Distilled water	100ml

The above ingredients were mixed and transferred into tubes in 5 ml amount and autoclaved.

12. Phenyl alanine deaminase test

Yeast Extract	3g
Dl-Phenylalamine	2 g
Disodium hydrogen phosphate	l g
Sodium Chloride	5 g
Agar	12g
Distilled water	1 lr

pH 7.4

Distributed in tubes and sterilized by autoclaving at 121° C for 1 5 minutes, allowed to solidify as long slopes.

13. Sugar fermentation medium

Peptone	15g
Andrade's indicator	10 ml
Sugar to be tested	20g
Water	1litre

Andrade's indicator is prepared from 0.5% aqueous acid fuchsin to which sufficient 1M sodium hydroxide has been added to turn the colour of the solution yellow.

Dissolve the peptone and Andrade's indicator in 1litre of water and add 20g of the sugar; sugars to be tested generally include glucose, sucrose,

lactose and maltose. Distribute 3ml amounts in standard test tubes containing an inverted Durham tube. Sterilize by steaming at 100 degree C for 30 min on 3 consecutive days.

14. Trypticase soy broth

Pancreatic digest of casein	17 gm,
Peptic digest of soyabean meal	3 gm
Sodium Chloride	5
Dipottasium hydrogen phosphate	2.5
Dextros	2.5
pH	7.3 $\pm$ 2

30 gm in 1000 ml of distilled water

15. Xylose lysine desoxycholate (XLD) agar

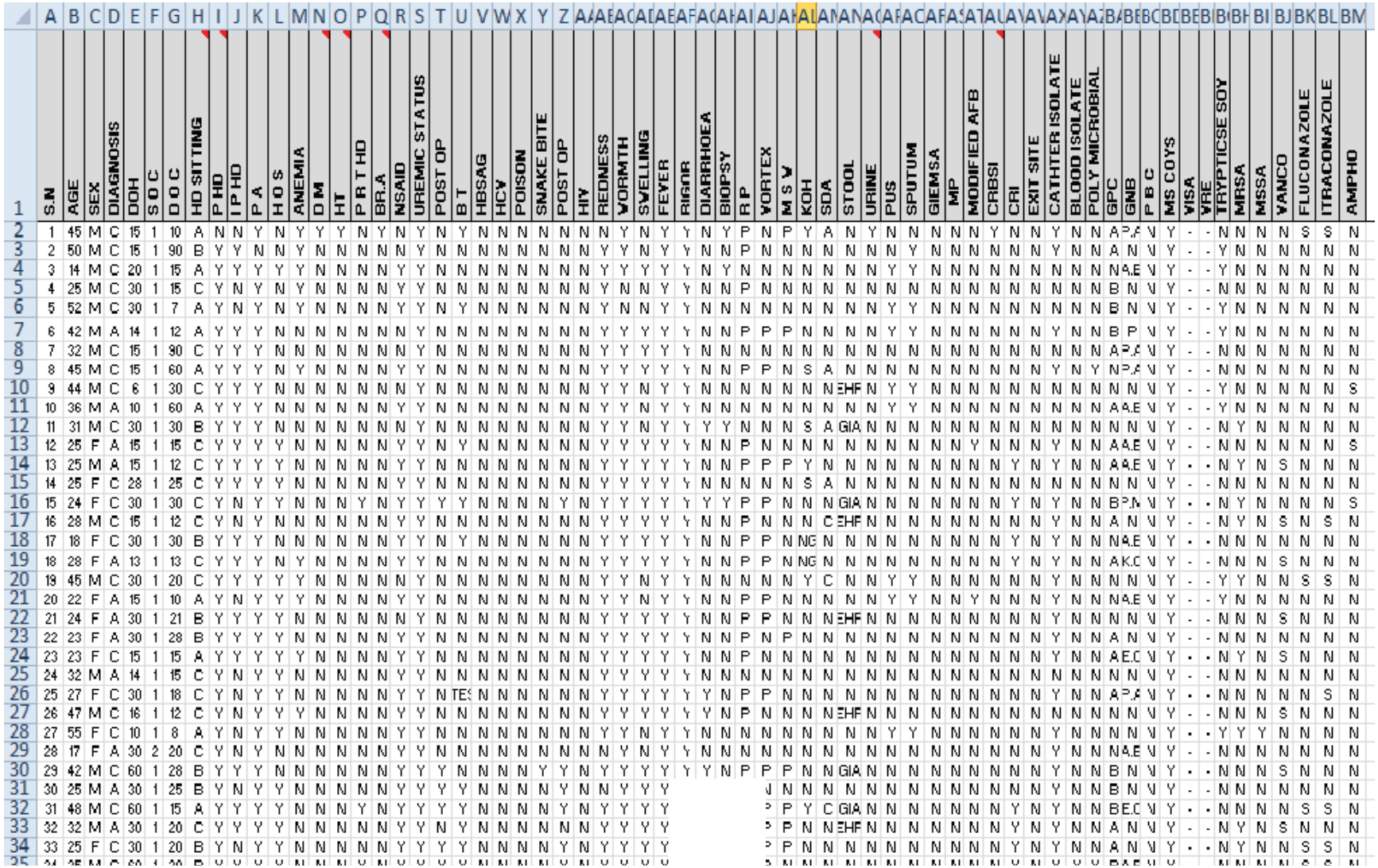
Yeast extracts	3 gm
Xylose	3.75 g
Lactose	7.5 gm
Sucrose	7.5 gm
L.Lysine HCL	5 gm
Sodium Chloride	5 gm
Sodium Deoxycholate	2.5 gm
Sodium Thiosulphate	6.8 gm
Ferric Ammonium Citrate	8 gm
Phenol red	indicator
Agar	15 gm

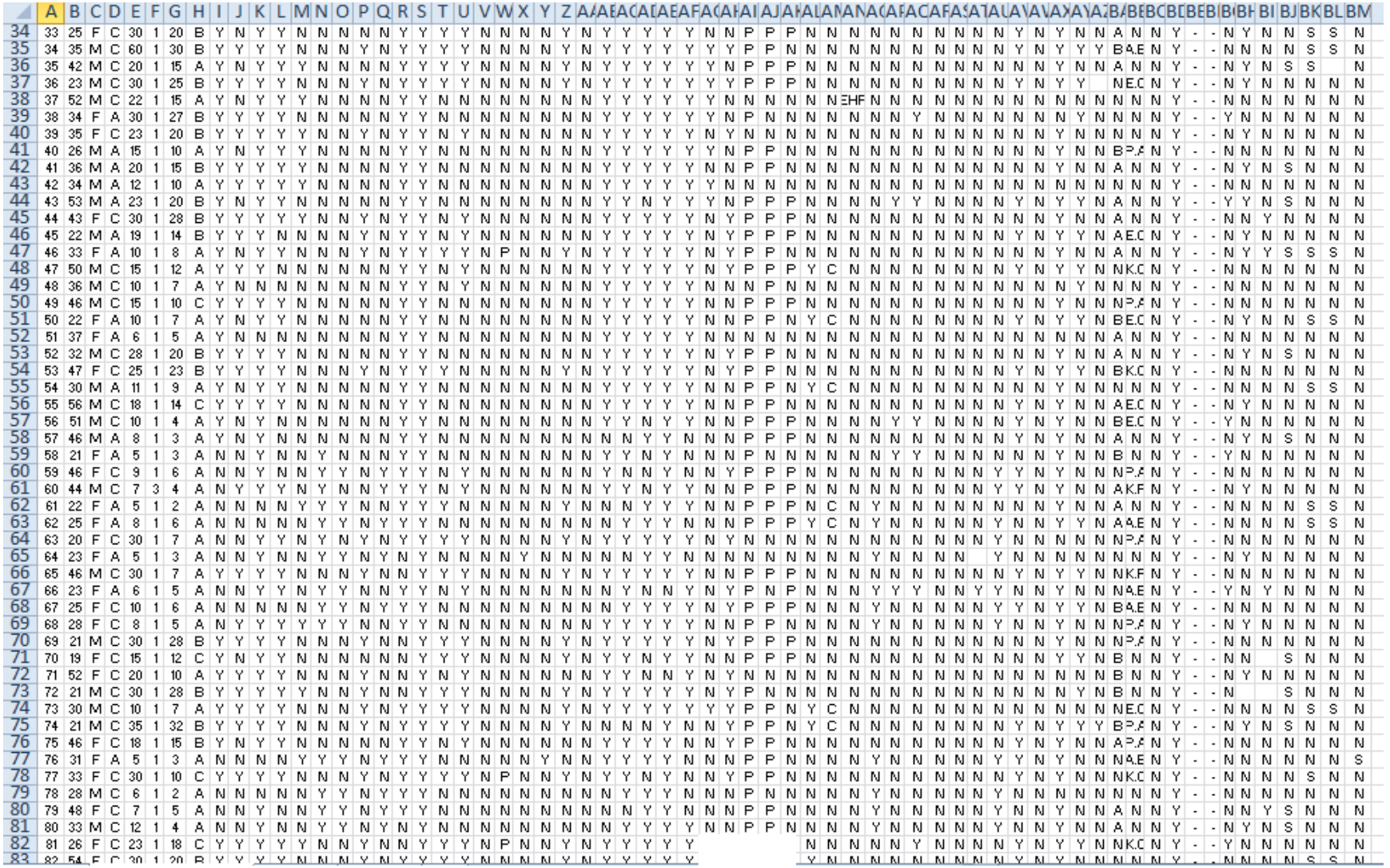
16. Deoxcholate citrate agar – DCA

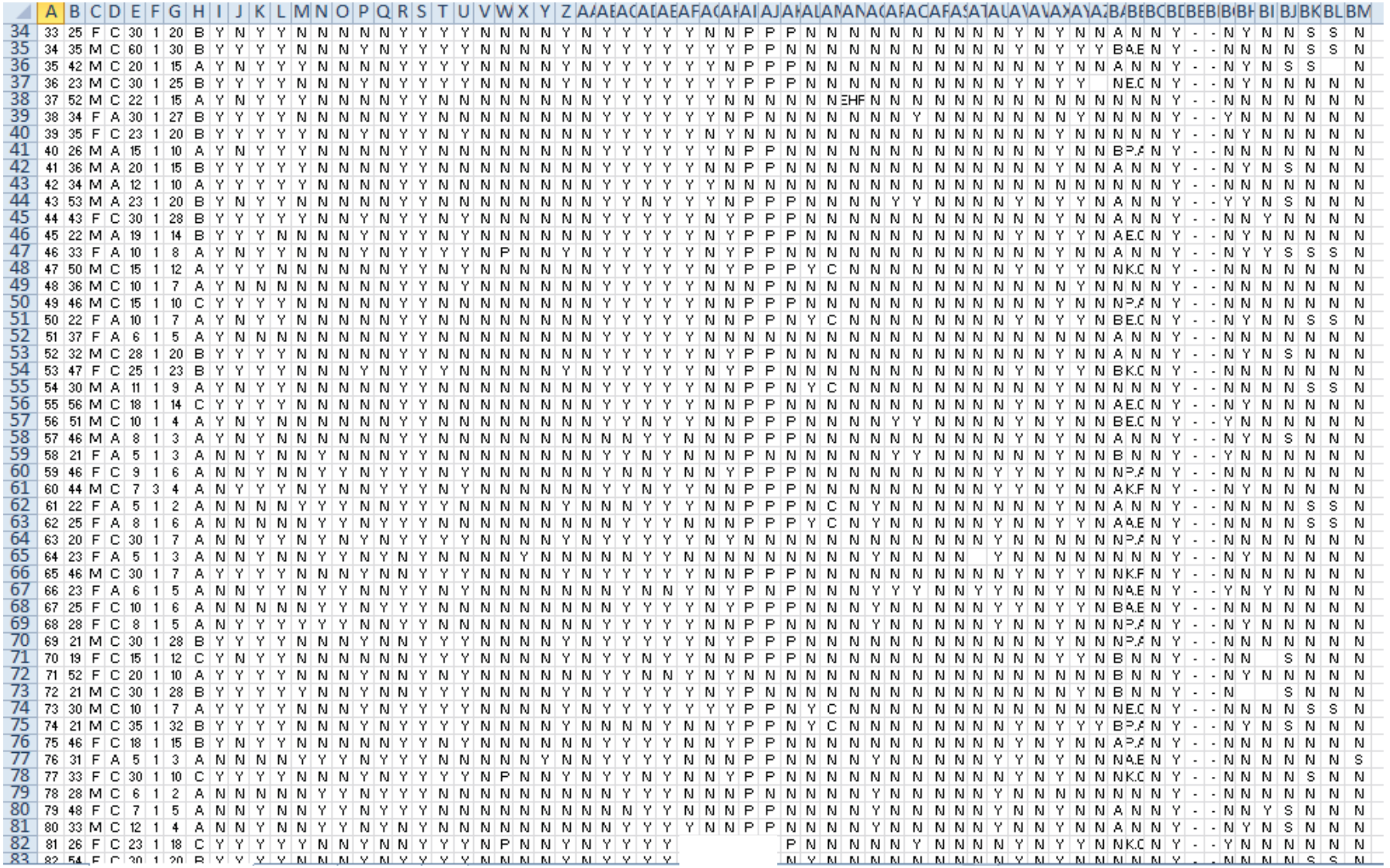
Meat Extract	20 mg
Peptone	20 gm
Neurtral ned	2%
in 50 ml ethanol	5 ml
Lactose	40 gm
Water	4 lt
pH	7.4

MASTER CHART KEY

M	-	Male
F	-	Female
Y	-	Yes
N	-	No
HOS	-	History of Steroids
DOC	-	Duration of Catheterization
HT	-	Hypertension
PRT-HD	-	Post Renal Transplant-HD
BA	-	Bronchial Asthma
NSAID	-	Non Steroidal Anti Inflammatory Drug
BT	-	Blood Transfusion
DOH	-	Duration of Hospitalization







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150	149	45	F	C	8	1	6	A	Y	N	N	N	N	N	N	N	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	-	-	N	N	N	N	N	N	N
151	150	29	M	C	10	1	8	C	Y	Y	Y	Y	Y	N	N	Y	N	N	Y	N	N	N	N	N	N	N	N	Y	Y	Y	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	-	-	N	N	N	N	N	N	N
152																																																																										
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